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Discovery of 5-substituted 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones as potent and selective Hsp90 inhibitors

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ABSTRACT

A new series of compounds, 5-substituted 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones, have been designed and identified as potent and selective inhibitors of Hsp90. These compounds demonstrated nanomolar potency toward both Hsp90-regulated Her2 degradation and the growth of a panel of human tumor cell lines in cell-based assays. High selectivity of these compounds toward Hsp90 was evident given that they did not inhibit a panel of 34 kinases at 10 μM. The structure–activity relationship (SAR) of this series is reported here.

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Hsp90 has attracted enormous attention and has emerged as a viable target for cancer therapy due to its critical roles in retaining the conformation and, hence, the function of its broad range of client proteins many of which are associated with cancer pathology.[1–3] Clinical proof of concept has been provided by a natural product Hsp90 inhibitor, 17-AAG which is under Phase III evaluation for the treatment of melanoma.[4,5] However, the side effects and poor formulability of 17-AAG restricted its potential for broad applications.[6] Small synthetic Hsp90 inhibitors emerged to address these issues presented by this natural product. Diverse chemotypes have been identified as potent Hsp90 inhibitors and advanced into clinics.[7–9] Most of these chemotypes bind to the N-terminal ATP binding site. It is interesting to note that different chemotypes have been found to induce quite unique conformational changes in Hsp90. This was confirmed by co-crystal structures of Hsp90-inhibitor complexes.[10–12]

Our knowledge-based design of Hsp90 inhibitors resulted in identification of an initial hit, 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-one (compound **1** in Fig. 1). The compound inhibited Hsp90 with an IC_{50} of 0.88 μM in a fluorescence polarization (FP) assay, induced the degradation of Hsp90 client protein Her2 with an IC_{50} of 0.57 μM, and inhibited the growth of human tumor cells including MCF-7, SKBR-3, BT474, and HT-29 with GI_{50} values less than 1.5 μM. It was anticipated that compound **1** binds to the N-terminal ATP binding site in a similar pattern to purine-based Hsp90 inhibitors.[7] The modeling study suggested later that the amino moiety at the 2-position of **1** interacts with D93 via the critical H-bond interaction, the pyridine ring at the 8-position interacts

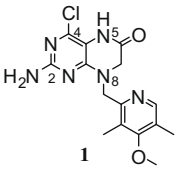

Figure 1. Knowledge-based design of 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-one (**1**) as an initial hit inhibiting Hsp90.

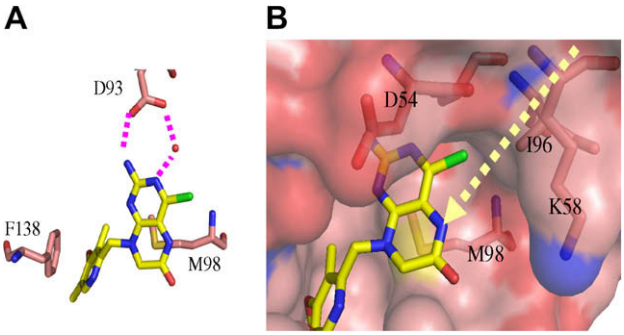

Figure 2. Docking of **1** in the N-terminal ATP binding site of Hsp90α. The model was built based on the Hsp90 structure (PDB code 3D0B). (A) 2-Amino group forms a hydrogen bond with D93 and nitrogen atom at the 3-position interacts with D93 via a water molecule (red ball). The pyridine ring forms π–π stacking with F138. (B) The 5-position directs toward the opening of the ATP binding site. The path from the 5-position to the opening (yellow arrow) is surrounded by the hydrophobic side chains of I96 and M98 as well as the carbon atoms of K58 and D54.

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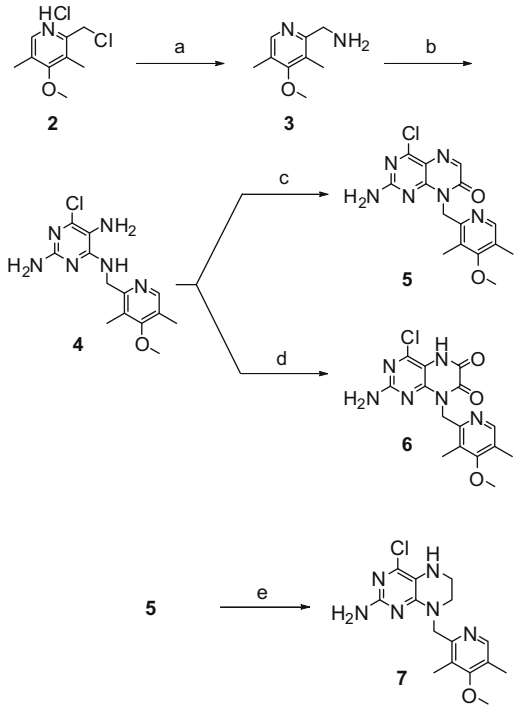
with F138 via π–π stacking, and the 7,8-dihydropteridin-6(5*H*)-one core interacts with M98 via hydrophobic interaction (Fig. 2A). The assessment of the pharmaceutical properties for **1** indicated that it has good aqueous solubility (381 μM at pH 3) and metabolic stability in human, rat, and mouse microsomes ($t1/2 > 60$ min). Most importantly, compound **1** demonstrated 60% bioavailability in vivo (rat). Compound **1** proved to be a viable hit for further structure elaboration to improve biological potency toward Hsp90. Here, we report the results of structure–activity relationship (SAR) studies on this new chemotype as a Hsp90 inhibitor.

Guided by the published co-crystal structure information and the modeling study, our medicinal chemistry efforts were focused on several positions of the core structure, 2-amino-7,8-dihydropteridin-6(5*H*)-one: (i) modification of the pteridinone ring, (ii) incorporation of diverse substitution, particularly solubilizing groups, at the 5-position, (iii) replacing the 4-Cl with other small moieties including methoxy, methyl, or other halogens, (iv) introduction of substitutions at the 7-position, (v) elaboration of aryl or heteroaryl rings at the 8-position.

The initial modification was focused on the pteridinone ring. It was found from the molecular modeling study that the carbonyl moiety at the 6-position was not found to be involved in any critical interaction (Fig. 2B). It was instead, directed toward the opening of the ATP binding site. Hence, various analogs with 7-carbonyl, 6,7-dicarbonyl or no substitution at either 6- or 7- position were prepared (Scheme 1).[13–16] The key intermediate, 6-chloro-N^4-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-pyrimidine-2,4,5-triamine (**4**), was prepared via condensation of 4,6-dichloropyrimidine-2,5-diamine and (4-methoxy-3,5-dimethylpyridin-2-yl)methan-amine (**3**) which was obtained via amination of 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (**2**). Condensation of **4** with ethyl 2-oxoacetate in the presence of acetic acid in toluene or with ethyl oxalyl chloride in acetone followed by refluxing in ethanol afforded 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)pteridin-7(8*H*)-one (**5**) and 2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)-methyl)-pteridine-6,7(5*H*,8*H*)-dione (**6**), respectively. 4-Chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-5,6,7,8-tetrahydropteridin-2-amine (**7**) was prepared by reduction of **5** with lithium aluminum hydride (LAH).

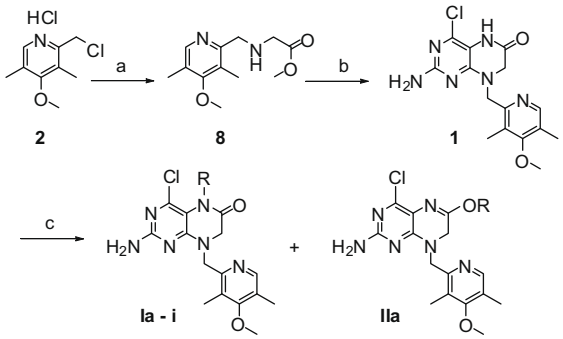


Scheme 1. Diversification of the pteridinone core. Reagents and conditons: (a) (i) K_2CO_3, DMF, isoindole-1,3-dione, room temperature, 24 h; (ii) NH_2NH_2, 50 °C, 1 h; 48% yield; (b) Et_3N, BuOH, 4,6-dichloropyrimidine-2,5-diamine, reflux, 3 h; 50% yield; (c) ethyl 2-oxoacetate, acetic acid, toluene, reflux, 4 h; 54% yield; (d) (i) ethyl oxalyl chloride, K_2CO_3, acetone, room temperature, overnight; (ii) Et_3N, EtOH, reflux, 4 h; 90% yield; (e) LAH, THF, room temperature for 60 min; 90% yield.

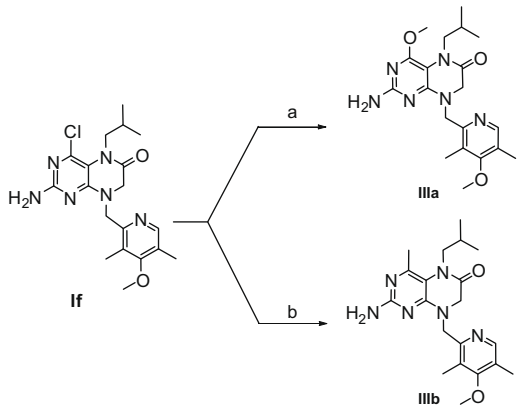
Based on the SAR results from the structural modifications of the pteridinone structure (compound **5**–**7**), the efforts were re-directed to **1** analog retaining 6-carbonyl group while functional groups at other positions were explored. From the modeling study, the substitution at the 5-position of **1** is directed toward the opening of the binding site and can be used as a handle to improve the pharmaceutical properties as well as potency. There is a hydrophobic environment between the 5-position and the opening of the binding site (Fig. 2B). Accordingly, various alkyl substitutions were first incorporated to explore the hydrophobic space around this position (Scheme 2). Solubilizing groups were then attached to some of the alkyl substitutions for improving pharmaceutical properties of these compounds. Compound **1** was prepared by condensation of 4,6-dichloropyrimidine-2,5-diamine with methyl 2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylamino)-acetate (**8**) which was obtained by amination of **2** with methyl 2-aminoacetate hydrochloride in ethanol. Alkylation of **1** with alkyl halides afforded the major N-alkylated product **Ia**–**i** and minor O-alkylated product **IIa**.[17] The ratio of the two products varied depending on the alkylating agents. Both **I** and **II** analogs were evaluated for their inhibitory potency toward Hsp90.

Modification at the 4-position was quite limited based on the modeling study indicating that the small space around this position may only accommodate substitutions like halogen, methyl, or methoxyl group. Compound **IIIa** and **IIIb** were prepared from **If** via direct displacement of the 4-Cl with a methoxyl and methyl group, respectively, under the condition described in Scheme 3.

The effect of adding a substituent at the 7-position was also elaborated by introducing various alkyl moieties (compounds **IVa**–**h** in Scheme 4). Compound **IVa** was synthesized via replacing the 4-Cl in 4,6-dichloropyrimidine-2,5-diamine with (*S*)-3-((4-methoxy-3,5-dimethylpyridin-2-yl)methylamino)-dihydrofuran-2(3*H*)-one (**9**) which was prepared via reductive amination using 4-methoxy-3,5-dimethyl-picolinaldehyde and (*S*)-3-aminodihydrofuran-2(3*H*)-one hydrochloride. Using the similar protocol, compound **IVb**–**e** (Scheme 4) were prepared with various substitutions at the 7-poisition. Alkylation of **IVc** and **IVd** at the 5-position using the same protocol as for the synthesis of **Ia**–**i** afforded compound **IVf**–**h**.



Scheme 2. Synthesis of 5-substituted-2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones. Reagents and conditions: (a) methyl 2-aminoacetate hydrochloride, K_2CO_3, EtOH, reflux, 1 day; 43% yield; (b) Et_3N, BuOH, 4,6-dichloropyrimidine-2,5-diamine, reflux, 18 h; 50% yield; (c) NaH, DMF, RX, room temperature, overnight.



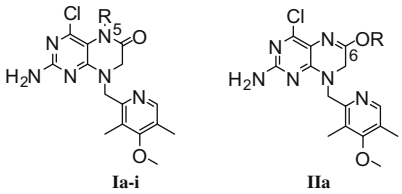
Scheme 3. Synthesis of 4-substituted-2-amino-5-isobutyl-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones. Reagents and conditions: (a) NaOH, CH_3OH–H_2O (6:1), reflux, 48 h; 70% yield; (b) $Pd(PPh_3)_4$, $AlMe_3$, THF, reflux, overnight; 50% yield.

Finally, the SAR study on the substituent at the 8-position was also elaborated by replacing substituted pyridines with other aromatic or heteroaromatic ring. The preparation of this series (**V**) followed the same procedure described for series **I** with aryl- or heteroaryl-methanamine replacing (4-methoxy-3,5-dimethylpyridin-2-yl)methan-amine.

All the analogs were first evaluated for their ability to induce degradation of the Hsp90 client protein Her2 in SKBR-3 cells. Selected compounds were then evaluated in a FP assay for their affinity to Hsp90. Compounds with IC_{50} values less than 2.0 μM in the Her2 degradation assay were then further evaluated for their inhibitory activity against the growth of several human tumor cell lines in culture, including MCF-7, BT474, SKBR-3, and HT29. To examine the selectivity at the cellular level, selected analogs were also assessed for cytotoxicity using a normal human mammary epithelial cell line (hMEpiC). The results are summarized in Tables 1–4.

To examine the importance of the 6-carbonyl group of **1**, several analogs were prepared by moving the carbonyl group from 6- to 7-position (**5**), adding an extra carbonyl at the 7-position (**6**), or eliminating this carbonyl group (**7**). All these modifications resulted in compounds with dramatically decreased potency in the Her2 degradation assay compared to the original compound **1** (Table 1). It is likely that the 6-carbonyl plays an important role (sterically or electronically) in the binding of these compounds to Hsp90. Further SAR was then focused on the modification at different positions of **1**.

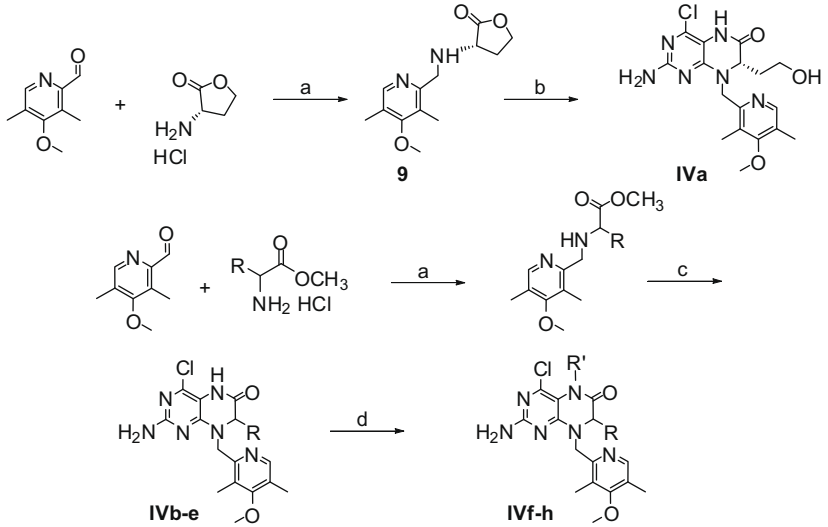
Table 1
In vitro biological activity toward Hsp90 in a FP and Her2 degradation assay



ID	R	FP assay IC_{50} [a] (μM)	Her2 degradation IC_{50} [a] (μM)
1	H	0.88 ± 0.16	0.57 ± 0.26
5	–	nt	>10
6	–	nt	>5
7	–	>5	>5
Ia	CH_3	0.96 ± 0.17	0.93 ± 0.23
Ib	$(CH_3)_2CH(CH_2)_3$–	0.90 ± 0.17	1.91 ± 0.58
Ic	(Pyrrolidin-1-yl)-$(CH_2)_2$–	0.66 ± 0.48	0.51 ± 0.29
Id	(Morpholino-1-yl)-$(CH_2)_3$–	nt	1.55 ± 0.34
Ie	$(CH_3)_2CH$–	0.07 ± 0.04	0.05 ± 0.03
If	$(CH_3)_2CHCH_2$-	0.05 ± 0.01	0.04 ± 0.01
Ig	Cyclopentyl	nt	0.11 ± 0.03
Ih	$(CH_3)_2N(CH_2)_2NHCOCH(CH_3)$-	nt	0.10 ± 0.02
IIa	Cyclopentyl	nt	0.01 ± 0.01
Ii	(Morpholin-1-yl)$(CH_2)_2NHCO$-	nt	>10

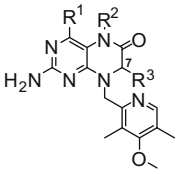
[a] The IC_{50} values are means of three independent experiments (nt = not tested).

Based on the modeling study, the path from the 5-position of **1** to solvent exposure at the opening of the ATP binding site of Hsp90 is surrounded by hydrophobic amino acid side chains of I96 and M98 as well as the carbon atoms of K58 and D54 (Figure 2B). Hence diverse alkyl (i.e., branched or straight chain) functionalities were introduced at the 5-position with the objective of increasing the affinity to Hsp90 via extra hydrophobic interactions. The solubilizing moiety was then attached to the alkyl groups at the 5-position



Scheme 4. Synthesis of 7-substituted-2-amino-4-chloro-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones. Reagents and conditions: (a) $NaBH(OAc)_3$, TEA, CH_2Cl_2, room temperature, 2 h; 57–76% yield; (b) 4,6-dichloropyrimidine-2,5-diamine, H_2SO_4, MeOH, reflux, overnight; 2.1% yield; (c) DIEA, *n*BuOH, 150 °C in sealed tube, 48 h; 5–10% yield; (d) NaH, DMF, RX., room temperature, overnight.

Table 2
Inhibition of Hsp90 binding and activity by 4,5,7-substituted 2-amino-8-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7,8-dihydropteridin-6(5*H*)-ones



IIIa-b and IVa-h

ID	R^1	R^2	R^3	FP assay IC_{50}[a] (μM)	Her2 degradation IC_{50}[a] (μM)
If	Cl	$(CH_3)_2CHCH_2$–	H	0.05 ± 0.01	0.04 ± 0.01
IIIa	OCH_3	$(CH_3)_2CHCH_2$–	H	nt	1.07 ± 0.30
IIIb	CH_3	$(CH_3)_2CHCH_2$–	H	nt	0.98 ± 0.06
IVa	Cl	H	(*S*)-CH_2CH_2OH	0.64 ± 0.07	0.28 ± 0.05
IVb	Cl	H	(*R*)-CH_3–	0.40 ± 0.06	0.35 ± 0.04
IVc	Cl	H	(*S*)-CH_3–	nt	0.43 ± 0.30
IVd	Cl	H	(*S*)-$(CH_3)_2CH$–	2.14[b]	0.65 ± 0.31
IVe	Cl	H	(*S*)-$(CH_3)_2CHCH_2$–	nt	0.37 ± 0.14
IVf	Cl	$(CH_3)_2CHCH_2$–	(*S*)-CH_3–	nt	>5
IVg	Cl	$(CH_3)_2CHCH_2$–	(*S*)-$(CH_3)_2CH$–	nt	>5
IVh	Cl	$(CH_3)_2CH$–	(*S*)-$(CH_3)_2CH$–	0.08 ± 0.01	0.06 ± 0.02

[a] The IC_{50} values are means of three independent experiments (nt = not tested).
[b] One experiment has been done.

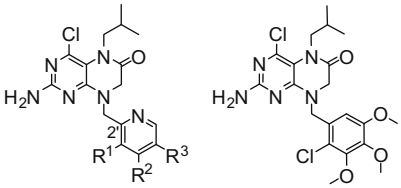
of active compounds to improve the solubility as well as other pharmaceutical properties. The potency of these compounds toward Hsp90 in a FP assay as well as their ability to induce Her2 degradation in SKBR-3 cells depended on the nature of the alkyl groups (Table 1). In this regard, an analog with a methyl group at the 5- position (**Ia**) gave an IC_{50} value of 0.93 μM in Her2 degradation assay similar to the potency of **1**. Compounds with a linear alkyl sub-unit attached to the 5-position showed comparable or slightly decreased potency (**Ib, Ic,** and **Id** vs **1**) in Her2 degradation assay. However, 5–14-fold increase in potency was observed in the Her2 degradation assay for compounds with an isopropyl (**Ie**), isobutyl (**If**), and cyclopentyl (**Ig**) moiety, implying the branched substitution is preferred at the 5-position. Based on the SAR conclusion, **Ih** with the solubilizing moiety attached to the branched substitution at the 5-position was prepared and demonstrated comparable potency to **Ie** in Her2 degradation assay. During the synthesis of the 5-alkylated analog, a minor O^6-alkylated product was also isolated and screened. Of particular interest, **IIa** induced Her2 degradation with an IC_{50} of 0.01 nM, over 11-fold more potent than its corresponding 5-alkylated isomer, **Ig**, indicating that both the 5- and 6-position could tolerate large substitutions which may offer additional hydrophobic interaction. Besides the 5-alkylated analogs, a compound with 5-urea substitution (**Ii**) lost the potency compared to **1**. There is, in general, a good correlation between the potency in the Her2 degradation and FP assay. Collectively, both the 5- and 6-position could tolerate diverse types of substitutions. Increased potency was observed for compounds with branched alkyl groups.

A structural modification at the 4-position of one of the most potent compound, **If** which has the optimal 5-isobutyl group, resulted in more than 24-fold decrease in potency in the Her2 degradation assay, implying that Cl is the optimal substitution at this position (**IIIa** and **IIIb** vs **If** in Table 2). Further medicinal chemistry efforts were focused on diversification at the 7-position in the presence of the 4-Cl group. Analogs of compound **1** with various alkyl or substituted alkyl groups at the 7-position (Table 2) demonstrated sub-micromolar potency comparable to **1** (i.e., **IVa–e**). The potency to induce Her2 degradation is not sensitive to the stereochemistry at the 7-position. **IVb** and its *S* isomer (**IVc**) showed similar potency. Of particular interest, introduction of the 7-alkyl substitution on **Ie** bearing 5-isopropyl moiety retained potency (**IVh** vs **Ie**) while the same modification on **If** with 5-isobutyl group greatly decreased potency (**IVf** and **IVg** vs **If**). This is consistent with previous SAR trend where the potency is sensitive to substitution at the 5-position. Overall, substitutions at the 7-position did not offer any advantage with regard to potency over the ones without such substituents. However, solubilizing moiety may be tolerable at this position with the right substituent at the 5-position.

Finally, the SAR study was directed to the pyridine ring while maintaining the optimal 4-Cl and 5-isopropyl or isobutyl substituents. Analogs with different substitutions on the pyridine ring or substituted phenyl replacing the pyridine ring were prepared and tested (Table 3). Replacing *para*-methoxy with Cl on the pyridine ring caused about 15-fold decrease in potency (**Va** vs **If**). However, a compound with a para-bromo substituent has only 2.5-fold decrease of potency in the Her2 degradation assay (**Vb** vs **If**). Analog with 3′,4′-dimethoxyl substituents on the pyridine ring (**Vc)** was found to be 4-fold less potent than **If**. Further more, replacing the pyridine with substituted phenyl ring (**Vd**) resulted in more than 125-fold decrease in potency in the Her2 degradation assay. Altogether, the SAR indicated that there is little room for the diversification on the aryl or heteroaryl (i.e., pyridine) ring. This is consistent with our molecular modeling study as well as previous SAR results on purine-based Hsp90 inhibitors,[17] which indicated the pyridine ring was positioned at the bottom of the ATP binding site (Fig. 2B).

Compounds active in the Her2 degradation assay were further evaluated for their ability to inhibit human tumor cell growth in vitro (Table 4). The sensitivity of these human tumor cells to this series of compounds is quite different. HT-29, SKBR-3, and BT474 cells were slightly more sensitive to the inhibitory activity of these analogs than MCF-7 cells. In general, there is a good correlation between the potency for the Her2 degradation and anti-proliferative activity. The most potent compounds in the Her2 degradation assay were also found to show high anti-proliferative activity with GI_{50} values ranging from 5 to 90 nM comparable to the anti-proliferative activity of geldenamycin. To assess the selectivity of these compounds toward tumor over normal cell growth, we have also tested selected compounds for their ability to inhibit growth of normal non-tumorigenic human mammary epithelial cells, hMEpiC. Compound **Ie** is 5–16-fold more selective for human tumor

Table 3
Inhibition of Hsp90 binding and activity by 8-(aryl-methyl) substituted 2-amino-4-chloro-5-isobutyl-7,8-dihydropteridin-6(5*H*)-ones



Va-c **Vd**

ID	R^1	R^2	R^3	FP assay IC_{50}[a] (μM)	Her2 degradation IC_{50}[a] (μM)
If	CH_3	OCH_3	CH_3	0.05 ± 0.01	0.04 ± 0.01
Va	CH_3	Cl	CH_3	nt	0.59 ± 0.19
Vb	CH_3	Br	CH_3	0.06 ± 0.01	0.10 ± 0.02
Vc	OCH_3	OCH_3	H	nt	0.16 ± 0.14
Vd	—	—	—	nt	>5

[a] The IC_{50} values are means of three independent experiments (nt = not tested).

Table 4
Growth inhibition of tumor cell lines and human mammary epithelial cell in vitro

ID	MCF-7 IC_{50}[a] (μM)	HT29 IC_{50}[a] (μM)	SKBR-3 IC_{50}[a] (μM)	BT474 IC_{50}[a] (μM)	hMEpiC IC_{50}[a] (μM)
1	1.49 ± 0.42	1.18 ± 0.52	0.39 ± 0.16	0.65 ± 0.10	nt
Ia	2.19 ± 1.28	1.47 ± 0.81	0.46 ± 0.08	0.95 ± 0.11	nt
Ib	3.11 ± 0.16	2.26 ± 0.69	0.97 ± 0.00	1.65 ± 0.22	nt
Ic	1.32 ± 0.35	1.08 ± 1.23	0.30 ± 0.26	0.52 ± 0.49	nt
Id	2.59 ± 0.71	2.95 ± 1.34	1.11 ± 0.15	1.81 ± 0.23	nt
Ie	0.05 ± 0.02	0.06 ± 0.06	0.02 ± 0.01	0.03 ± 0.03	0.32 ± 0.21
If	0.07 ± 0.03	0.04 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.13 ± 0.08
Ig	0.82 ± 0.35	0.33 ± 0.06	0.12 ± 0.01	0.29 ± 0.04	nt
Ih	0.12 ± 0.03	0.10 ± 0.03	0.03 ± 0.005	0.06 ± 0.01	nt
Ii[b]	2.14	1.20	>5	>100	nt
IIa	0.04 ± 0.02	0.02 ± 0.01	0.005 ± 0.004	0.01 ± 0.003	nt
IVb	0.79 ± 0.24	0.47 ± 0.04	0.22 ± 0.05	0.41 ± 0.01	nt
IVc	0.94 ± 0.15	0.66 ± 0.46	0.25 ± 0.11	0.47 ± 0.20	nt
IVd	1.60 ± 0.41	1.06 ± 0.32	0.43 ± 0.09	0.76 ± 0.17	nt
IVe	0.93 ± 0.07	0.69 ± 0.45	0.23 ± 0.02	0.49 ± 0.07	nt
IVh	0.04 ± 0.04	0.09 ± 0.08	0.005 ± 0.004	0.02 ± 0.01	nt
Va	0.47 ± 0.26	0.28 ± 0.07	0.11 ± 0.02	0.24 ± 0.03	nt
Vb	0.28 ± 0.21	0.14 ± 0.03	0.06 ± 0.01	0.13 ± 0.02	0.18 ± 0.12
Vc	0.10 ± 0.06	0.04 ± 0.01	0.01 ± 0.00	0.03 ± 0.003	0.24 ± 0.15

[a] The IC_{50} values are means of three independent experiments (nt = not tested).
[b] One experiment was done.

cells tested than normal cells. Comparing to its growth inhibition on hMEpiC, compound **If** is highly selective for SKBR-3 (13-fold) and BT474 (6-fold) but less selective for MCF-7 (2-fold) and HT29 (3-fold). The same selectivity trend was seen for compound **Vc** which is 6–24-fold selective for SKBR-3, BT474 and HT29 but only 2-fold for MCF-7. Even though, the selectivity data will help us prioritize compounds for further evaluation, the mechanisms underlying the differential selectivity of these compounds are not quite understood. Furthermore, the implication of the cellular selectivity in in vivo setting is still remained to be revealed.

In summary, we have reported a new series of Hsp90 inhibitors which demonstrated high potency in a FP, Her2 degradation, and tumor cell growth assay in vitro. It was anticipated that these compounds would bind to the N-terminal ATP binding site of Hsp90 similar to other purine-based Hsp90 inhibitors. Both SAR and molecular modeling studies supported this mechanism of action. Co-crystal structures will further refine our understanding of the molecular mechanisms by which these compounds inhibit Hsp90. These compounds are highly selective for Hsp90 over 34 tyrosine or serine-threonine kinases (data not shown). They did not inhibit the activity of these kinases at 10 μM. At the cellular level, some compounds also selectively inhibit the growth of human tumor cells over normal human cells. Efforts are currently directed toward improving the in vivo properties of these analogs with regard to their pharmacokinetic properties and efficacy.

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