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Brief Article

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Dihydroxyphenylisoindoline Amides as Orally Bioavailable Inhibitors of the Heat Shock Protein 90 (Hsp90) Molecular Chaperone†

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The discovery and optimization of potency and metabolic stability of a novel class of dihyroxyphenylisoindoline amides as Hsp90 inhibitors are presented. Optimization of a screening hit using structure-based design and modification of log *D* and chemical structural features led to the identification of a class of orally bioavailable non-quinone-containing Hsp90 inhibitors. This class is exemplified by **14** and **15**, which possess improved cell potency and pharmacokinetic profiles compared with the original screening hit.

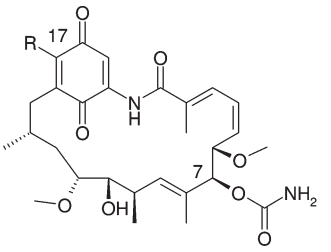
Introduction

Molecular chaperones are protein machines that are responsible for the correct folding, stabilization, and function of other proteins in the cell.[1] Among all the molecular chaperones, a 90 kDa heat shock protein (Hsp90[a]) has became an exciting target for cancer therapy.[2] Overexpression of Hsp90 in solid and hematologic tumors also suggests a role for the chaperone in oncogenesis.[3] The known Hsp90 antibiotics include the N-terminus ATP-competitive binders (such as geldanamycin (GA) and radicicol[4]) and the C-terminus ATP-site binder, such as novobiocin and its derivatives.[5] Compound **1a** (GA) and its 17-substituted semisynthetic analogues (17-allylaminogeldanamycin (17-AAG), 17-dimethylaminogeldanamycin (17-DMAG), and 17-propylaminogeldanamycin (17-PGA), Figure 1) were reported in the literature over the past decade. **1a** and **1b** (17-AAG) display efficacy in preclinical tumor models but suffer from poor solubility,[6a] limited bioavailaility,[6b] hepatotoxicity,[6c] and extensive metabolism by polymorphic enzymes.[6d] These liabilities complicate further pharmaceutical development of these agents. The undesirable chemical features associated with **1a** and its analogues (quinone moiety, relatively high molecular weight) have led to siginificant efforts to identify novel non-quinone-containing small molecule inhibitors of Hsp90. NVP-AUY922,[7] BIIB021,[8] and SNX5422[9] (Figure 2) are recent examples of such inhibitors that were identified by high throughput screening. They are currently in phase I/II, phase II, and phase I trials, respectively, and their preclinical pharmacokinetic and efficacy results have been reported in the literature.[7−9]

Result and Discussion

Our Hsp90 research efforts led to the initial discovery of dihydroxylphenylpyrrolidine **2a** (Table 1) which displayed reasonable competitive binding properties (IC_{50} of 20 nM) and an IC_{50} of 1 μM as assessed by the degradation of Hsp90 client protein, Akt, in H1299 cells.[10] This compound was stable in a human liver microsome assay (HLM)[11] but was unstable in a human hepatocyte metabolism assessment (hHep). The instability in the hHep assay of **2a** was identified by mass difference from the parent compound and is due to phase II conjugation of the phenolic groups present in the molecule.[12] Similar in vitro and in vivo instability was observed for a related resorcinol-containing Hsp90 inhibitor previously described in the literature.[7] Therefore, the main priorities of the lead optimization effort associated with this dihydroxyphenyl class of inhibitor were to improve Hsp90 inhibitory potency and compound stability in the hHep assay.

Our first laboratory objectives were to improve the cellular inhibitory potency of **2a** and to explore the correlation between predicted lipophilicity (clogD) of related compounds and their clearance[13] in the hHep assay. Compound **3** (Table 1) reduced lipophilicity by 2 log units relative to **2a**



1a, GA: R=OCH_3
1b, 17-AAG: R=-$NHCH_2CH{=}CH_2$
1c, 17-DMAG: R=-$NHCH_2CH_2NMe_2$
1d, 17-PGA: R=-$NHCH_2CH_2CH_3$

Figure 1. Structures of quinone ansamycins (**1a**−**d**).

†Coordinates of Hsp90 complexes with **2a**, **2b**, and **15** have been deposited in the Protein Data Bank under accession codes 3K97, 3K99, and 3K98.

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[a] Abbreviations: Hsp90, 90 kDa heat shock protein; 17-AAG, 17-allylamino-17-demethoxygeldanamycin; 17-DMAG, 17-dimethylamino-17-demethoxygeldanamycin; 17-PGA, 17-propylamino-17-demethoxygeldanamycin; HTS, high throughput screening; EtOAc, ethyl acetate; NaOAc−HOAc, sodium acetate and acetic acid; DMSO-d_6, hexadeuteriodimethyl sulfoxide; DCM, dichloromethane; MeOH, methanol; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; LC/MS, liquid chromatogrpahy−mass spectrametry.

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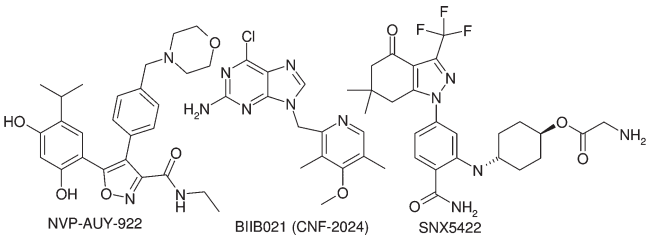
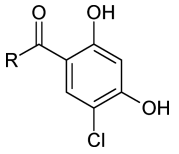
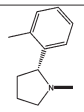
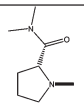
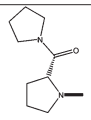
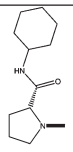


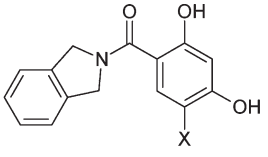
Figure 2. Structures of known Hsp90 inhibitors.

Table 1. Relationship between Lipophilicity and in Vitro Human Hepatocyte Clearance



#	R	Enzyme Ki[a] (nM)	H1299 Cell IC_{50} (μM)	clog D	CL_{hHep} (μL/min) / million cells
2a		10	1	2.9	129
3		260	18%@ 10 μM	0.8	56
4		400	10%@ 10 μM	-0.3	<7
5		200	24%@ 10 μM	0.6	<9
6		182	10%@ 10 μM	1.4	9

[a] Enzymatic K_i was calculated from enzymatic IC_{50} using Cheng–Prusoff equation.



2b: X=H; Ki= 60 nM; H1299 cell IC_{50} =7 μM
2c: X=Cl; Ki= 10 nM; H1299 cell IC_{50} =1 μM

Figure 3. Hsp90 isoindoline lead structures.

by removing the 2-methylphenyl moiety (from 2.9 to 0.8) but did not reduce the corresponding clearance in the hHep assay. Compounds **4**, **5**, and **6** were designed to introduce an amide moiety to occupy a hydrophobic Hsp90 pocket (formed by Gly135, Val136, and Tyr139) that was described in a previous publication.[10] These inhibitors displayed reduced lypophilicity

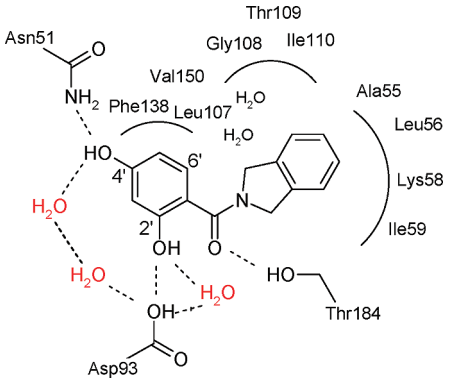


Figure 4. Schematic diagram showing the key interactions of **2b** with Hsp90 enzyme. Water molecules in red represent structurally conserved water molecules.

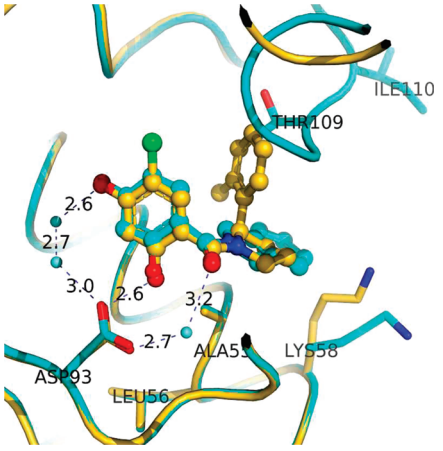
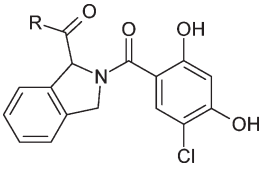


Figure 5. Overlap of the cocrystal structures of **2a** and **2b** with human Hsp90 enzyme.

Table 2. SAR of 5-Chloro-2,4-dihydroxyphenylisoindoline-1-carboxyamide

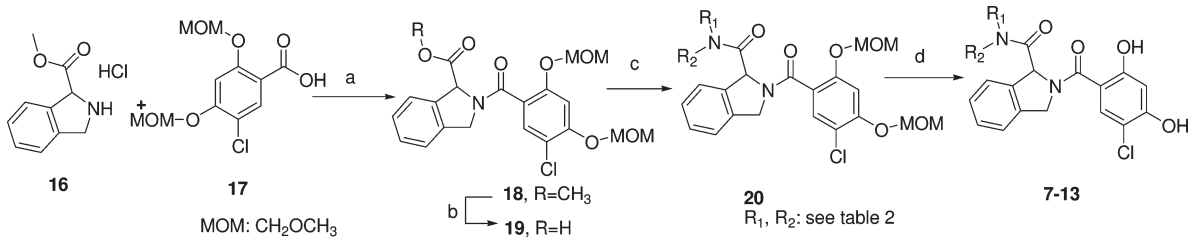


compd	chirality	R	K_i[a] (nM)	cell[b] IC_{50} (μM)	clogD	CL_{hHep} (μL/min)/ million cells
7	*R*/*S*	$N(CH_3)_2$	20	1.5	0.7	2.4
8	*R*/*S*	$N(CH_2)_4$	10	0.6	1.6	2
9	*R*/*S*	$NH(CH_2)_2CH_3$	7	1.5	1.4	9
10	*R*/*S*	$NHCH_2CH_3$	4	0.3	0.9	4.7
11	*R*/*S*	$NHCH_3$	40	1.7	0.4	<2
12	*R*/*S*	$NHCH_2CH{=}CH_2$	5	1.5	1.2	8.5
13	*R*/*S*	$NHCH_2Ph$	20	0.7	2.2	25
14	*R*	$N(CH_2)_4$	4	0.6	1.6	11
15	*R*	$NHCH_2CH_3$	<1	0.3	0.9	7
1c			250	0.1	0.6	<3

[a] Enzyme K_i. [b] H1299 cell.

relative to **2a**. Surprisingly, **6**, which has a similar clogD compared with **3**, improved the in vitro clearance from 56 to 9 (μL/min)/million cells. Compounds **4**, **5**, and **6** (Table 1) all displayed reduced in vitro clearance compared with **2a** and **3**

Scheme 1. General Synthesis of the 2-(5-Chloro-2,4-dihydroxybenzoyl)-2,3-dihydro-1*H*-isoindole-1-carboxylic Amides[a]



[a] Reagents and conditions: (a) 4-methylmorpholine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF, 23 °C, 12 h, 64%; (b) NaOH(aq) (2 M), MeOH, 23 °C, 12 h, 94%; (c) various amines (both commercially available or synthetic amines), 4-methylmorpholine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole, DMF, 23 °C, 12 h, 64.2%; (d) HCl (in dioxane, 4 M), DCM, 23 °C, 12 h, 82%.

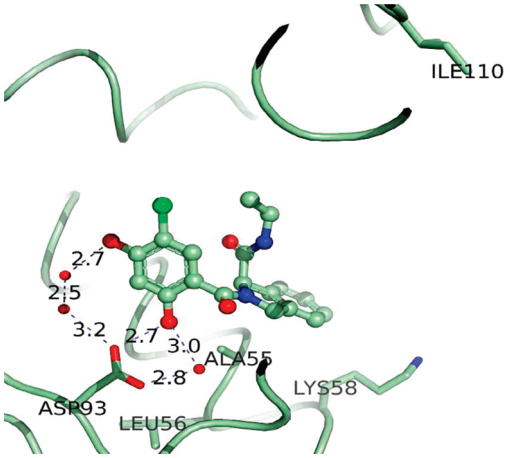


Figure 6. Crystal structure of **15** with human Hsp90 enzyme.

and possessed clogD values from −0.3 to 1.4. We therefore speculate that **4**−**6** contain a unique structural feature that reduces the glucuronidation of the associated resorcinol phenolic moieties.

To facilitate potency improvements of **4**−**6**, we sought to gain additional interactions with the Hsp90 enzyme. Another previously discovered HTS hit, **2b** (Figure 3), was used to assess the possibility of improving the inhibitors' potency through gaining different interactions with the Hsp90 enzyme. A chlorine atom was introduced next to the para-hydroxy group to confirm a SAR similar to that observed for the previous dihydroxyphenylpyrrolidine series.[10,14] Compound **2c** (Figure 3) displayed a competitive binding IC_{50} of 20 nM, a cellular IC_{50} of 1 μM, and high clearance in the hHep assay (93 (μL/min)/million cells). These results provided evidence that the two classes of inhibitors (pyrrolidine and isoindoline) shared the same SAR derived from occupying the small Hsp90 hydrophobic pocket formed by Leu107, Val150, and Phe138 (Figure 4). We also obtained a 2.1 Å cocrystal structure of **2b** bound to Hsp90. In the overlap of the cocrystal structures of **2a** (PDB code 3K97) and **2b** (PDB code 3K99), Lys58 was displaced by the phenyl portion of the isoindoline moiety present in **2b** to form a new hydrophobic pocket formed by Lys58, Leu56, and Ala55 (Figure 5). In addition, three other Hsp90 residues that surrounded the isoindoline moiety (Ile110, Thr109, and Gly108) were pushed away by the phenyl group of **2a** (Figure 5). Compounds **7** and **8** were then designed based on **4** and **5** to occupy this space. Compounds **7** and **8** improved the binding affinity 20-fold relative to **4** and **5**, respectively, and exhibited cell potencies of 0.6−1.5 μM.

Table 3. Rat PK Profiles of Two Compounds

	14	**15**
In Vitro		
hHep CL ER	0.2	0.3
rat Hep CL ER	0.2	0.2
In Vivo		
Cl ((L/h)/kg)	1.4	1.4
$AUC_{0\text{-inf}}$ (μg·h/mL) (po) (10 mg/kg)	2.9	1.3
$AUC_{0\text{-inf}}$ (μg·h/mL) (iv) (2.5 mg/kg)	2.1	1.7
V_{dss} (L/kg)	8.1	3.3
$T_{1/2}$ (h)	7.9	5.2
F (%)[a]	35	20

[a] $F_{oral} = (dose_{iv} \times AUC_{po})/(dose_{po} \times AUC_{iv})$.

Table 4. Mouse Plama Levels of Compound **15** through Different Administration Routes

adm route	iv	ip	po
dose (mg/kg)	20	100	100
$T_{1/2}$ (h)	5.2		8.3
free plasma concn at 1 h (nM)	286	16000	810
free plasma concn at 4 h (nM)	76	410	330

Several other amides were designed and synthesized to further elucidate the relationship between lipophilicity and clearance in the hHep assay for this class of compounds. These 2,3-dihydro-1*H*-isoindole-1-carboxylic acid amides (**7**−**13**, Table 2), which have clogD between 0.4 and 1.6, displayed reasonable clearance values in the hHep assay. Compound **13** has a higher clogD value (2.2) than the other amides and displayed higher clearance (> 10 (μL/min)/million cells). This result demonstrated that the 2,3-dihydro-1*H*-isoindole-1-carboxylic acid amides, which have a clogD of 0.4−2.2, could reduce the binding of the resorcinol-containing compounds to the uridine glucuronosyltransferase (UGT) enzymes.

Dihydroxylphenylpyrrolidine **2a** was prepared following the route previously described.[10] Compounds **3**−**6** were prepared following the same amide coupling procedure using commercially available D-proline methyl ester hydrochloride. Compounds **7**−**13** were prepared using key intermediates **16**[15] and **17**[10] as shown in Scheme 1.

The active enantiomers of the two most potent compounds **14** and **15** were isolated from their racemic counterparts **8** and

10, respectively, and further tested in rat PK experiments.[16] A cocrystal structure of **15** and Hsp90 was obtained (Figure 6, PDB code 3K98) and confirmed that the carboxylamide moiety occupied the same space as the 2-methylphenyl moiety exists in **2a**. Both **14** and **15** displayed a good correlation between in vitro and in vivo hepatic clearances in rat along with acceptable oral bioavailabilities (Table 3). We also examined **15** in a mouse PK experiment using different adminstration routes (Table 4). Both ip and po routes afforded compound plasma levels above the in vitro cellular IC_{50} for up to 4 h. In comparison, iv administration of **1c** to mice (6 mg/kg) afforded a $T_{1/2}$ of 1.8 h.

On the basis of these experiments, we identified the dihyroxybenzoyl 2,3-dihydro-1*H*-isoindole-1-carboxylic acid amides as bioavailable small molecule inhibitors of the Hsp90 target with the potential to display therapeutic effects.[17]

Conclusion

In summary, we have identified a series of dihydroxyphenylisoindoline amide compounds as potent, metabolically stable, and orally bioavailable non-quinone Hsp90 inhibitors. The process of lead optimization utilized structure-based drug design techniques, and the exploration between compound lipophilicity and phase II conjugation metabolism is also discussed. Additional results from our Hsp90 program will be reported in the near future.

Experimental Section

2-(5-Chloro-2,4-bis-methoxymethoxybenzoyl)-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid Methyl Ester (18). 4-Methylmorpholine (39 mL, 351 mmol), 1,(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (9 g, 47 mmol), and 1-hydroxybenzotrizole (HOBt) (6.3 g, 47 mmol) were added to a solution of **16** (5 g, 20 mmol) and **17** (6.5 g, 23.4 mmol) in DMF (100 mL). The mixture was stirred at room temperature for 12 h. Water (100 mL) was added to the mixture, and EtOAc (2 × 300 mL) was added to extract the aqueous solution. The combined organic layer was dried, filtered, and concentrated to get a brown oil. The crude product was purified by silica gel chromatography (eluting with 30% EtOAc in hexanes) to afford compound **18** (6.4 g, 60% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.69 (s, 3 H), 4.71−4.89 (m, 2 H), 5.67−5.76 (m, 1 H), 6.61 (s, 1 H), 7.16 (s, 1 H), 7.24−7.53 (m, 4 H), 10.34 (s, 1 H), 10.51 (s, 1 H). Anal. Calcd for $C_{17}H_{14}NO_5Cl \cdot 0.25H_2O$: C, 57.97; H, 4.15; N, 3.98. Found: C, 58.06; H, 4.17; N, 4.00.

2-(5-Chloro-2,4-bis-methoxymethoxybenzoyl)-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid (19). Lithium hydroxide (73.4 mL, 4 M, 294 mmol) was added to a solution of **18** (6.4 g, 15 mmol) in dioxane (30 mL). The mixture was heated at 60 °C for 3 h and monitored by LC/MS. The solvent was evaporated and acidified with NaOAc−HOAc buffer. EtOAc (2 × 200 mL) was added to extract the aqueous solution. The combined organic layer was dried, filtered, and concentrated to give **19** as a light-brown foam (6.3 g, quant. yield). Compound **19** was used in the next step without further purification.

2-(5-Chloro-2,4-dihydroxybenzoyl)-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid Ethylamide (10). 4-Methylmorpholine (9.2 mL, 83. 4 mmol), dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (6.4 g, 33.4 mmol), and 1-hydroxybenzotriazole (4.5 g, 33.4 mmol) were added to a solution of **19** (6.3 g, 17 mmol) and ethylamine (25 mL, 2 M in THF) in DMF (100 mL). The mixture was stirred at room temperature for 12 h and monitored by LC/MS. Water (50 mL) was added to the mixture, and EtOAc (2 × 300 mL) was added to extract the aqueous solution. The combined organic layer was dried, filtered, and concentrated to get a brown oil. This oil residue (**20**) was used in the next step without further purification.

Hydrogen chloride (42 mL, 4 M in dioxane, 167 mmol) was added to a solution of **20** in DCM (20 mL). MeOH was added, and the mixture was stirred at room temperature for 12 h. The solvents were evaporated. Water (50 mL) was added, and the pH of the resulting solution was adjusted to 7 by the careful addition of saturated $NaHCO_3$(aq) to basicify the aqueous solution. EtOAc (2 × 100 mL) was added to extract the aqueous solution. The combined organic layer was concentrated to get a brown oil. DCM (20 mL) was added and the pink precipitate was collected to afford the desired **10** (5.2 g, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01 (t, J = 7.2 Hz, 3 H), 2.82−2.91 (m, 1 H), 3.08−3.22 (m, 1 H), 4.62−4.90 (m, 2 H), 5.55 (s, 1 H), 7.22−7.35 (m, 4 H), 7.40 (d, J = 7.3 Hz, 1 H), 8.07 (s, 1 H), 10.06 (s, 1 H), 10.42 (s, 1 H). Anal. Calcd for $C_{18}H_{17}N_2O_4Cl$: C, 59.92; H, 4.75; N, 7.76. Found: C, 59.93; H, 4.84; N, 7.62.

(*R*)-2-(5-Chloro-2,4-dihydroxybenzoyl)-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid Ethylamide (15). Two enantiomers of **10** (5 g) were separated by supercritical fluid chromatography (SFC) using Chiralpak AD-H SFC column (21.2 mm × 250 mm) and eluted with 25% MeOH in carbon dioxide at 120 bar (flow rate at 60 mL/min). Two peaks that have retention times of 4.89 and 6.05 min were separated. The peak with the retention time of 4.89 min was identifed to be **15** (the active enantiomer) and to be the "*R*" enantiomer based on the crystal structure (Figure 6). Compound **15** displayed chiral purity of >95% ee.

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Supporting Information Available: Experimental details and spectral data for all compounds other than **18**, **19**, **10**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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