Bioorganic & Medicinal Chemistry Letters 21 (2011) 2278–2282

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and SAR of macrocyclic Hsp90 inhibitors with increased metabolic stability and potent cell-proliferation activity

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ARTICLE INFO

Article history:
Received 12 January 2011
Revised 23 February 2011
Accepted 24 February 2011
Available online 28 February 2011

Keywords:
Hsp90 inhibitors
Chaperon inhibitors
ortho-Aminobenzamides
N-terminal ATP-binding site
Macrocycles
Buchwald–Hartwig cyclization

ABSTRACT

A novel series of macrocyclic *ortho*-aminobenzamide Hsp90 inhibitors is reported. A basic nitrogen within the tether linking the aniline nitrogen atom to a tetrahydroindolone moiety allowed access to compounds with good physical properties. Important structure–activity relationship information was obtained from this series which led to the discovery of a soluble and stable compound which is potent in an Hsp90 binding and cell-proliferation assay.

Modern cancer chemotherapy generally suffers from the emergence of drug resistance.[1,2] The selective silencing of a specific cell signaling pathway by a chemotherapeutic is arguably at the origin of this problem. By up-regulating alternative pathways, tumors are able to adapt to the drug treatment and continue their growth. Targeting chaperone proteins offers a promising solution to this traditional chemotherapy dilemma since it allows the simultaneous inhibition of multiple pathways with a single agent.[3,4]

The ATP-dependent 90 kDa molecular chaperone Hsp90 has become an attractive target for cancer therapy.[5,6] It plays a critical role in maintaining the function of a wide range of client proteins many of which are intimately involved in cancer pathology.[7] Inhibition of Hsp90 leads to the destabilization and ultimately degradation of the clients which results in the inhibition of cell growth and apoptosis.[8]

Different ATP-competitive chemotypes have evolved as potent *N*-terminal Hsp90 inhibitors, several of which have transitioned into clinical trials.[9–11] While many of these clinical trials focus on Hsp90 inhibitors as single agents for cancer therapy, combination studies with established chemotherapeutics have been designed and were reported recently as well.[12]

The first Hsp90 inhibitor reported in the literature was the macrocyclic natural product Geldanamycin 1 (Fig. 1) which belongs to the class of the ansamycins.[13] Clinical studies established this compound's unacceptable toxicology profile preventing it from further development.[14] Optimization studies led to the discovery of 17-AAG **2** and 17-DMAG **3**[15] which are currently in clinical trials but which are characterized by poor solubility and a narrow therapeutic window, respectively.

Vernalis recently disclosed resorcinol **4**[16] which entered clinical trials in 2007. Isoxazole **4** is one of many small molecules which have been described in the literature as potential novel Hsp90 inhibitors with clinical application and is part of a recent summary on this topic.[17]

Serenex (acquired by Pfizer) recently disclosed their own efforts to discover potent small-molecule Hsp90 inhibitors that would be devoid of drawbacks associated with Geldanamycin.[18] Their studies culminated in a series of 2-aminobenzamides which exhibited low-nanomolar potencies in a proliferation assay. Among the reported compounds, glycine pro-drug SNX-5422 **5** was forwarded to clinical trials.[19]

Using structure-based drug design, a series of potent benzisoxazoles as Hsp90 inhibitors was recently discovered.[20] In continuation of seeking potent small molecule inhibitors of Hsp90 and guided by X-ray crystallography using a structural analog of **5**,

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doi:10.1016/j.bmcl.2011.02.101

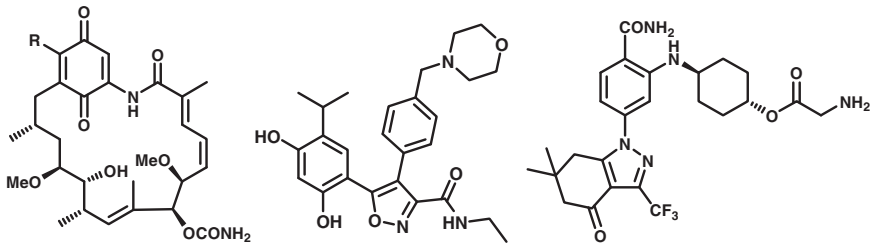


Figure 1. Structure of Geldanamycin **1**, clinically relevant ansamycins 17-AAG **2**, 17-DMAG **3**, Vernalis' clinical candidate NVP-AUY922 4 and Serenex' clinical candidate SNX-5422 5.

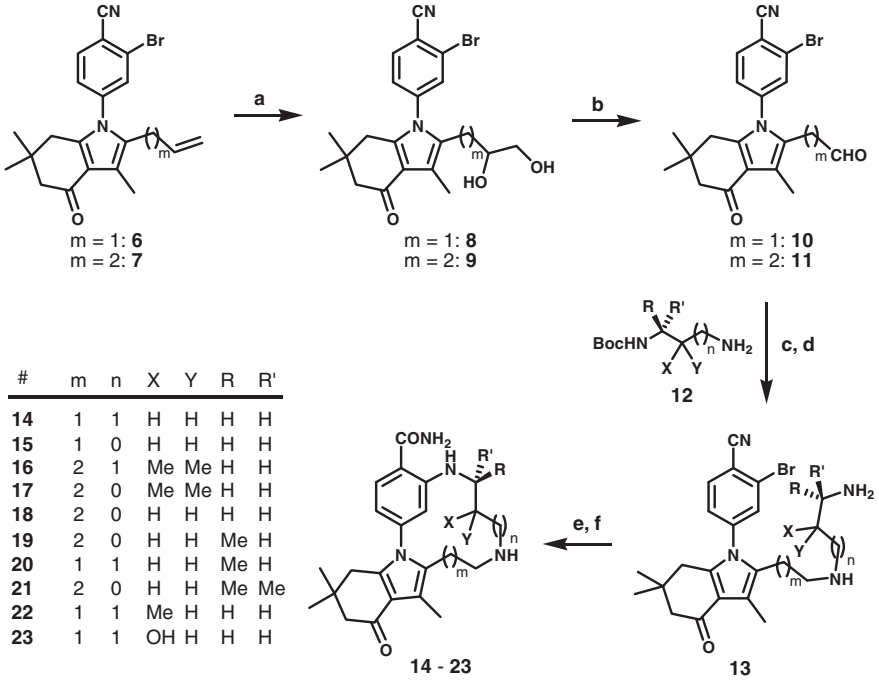
we were able to design structurally unique macrocyclic *ortho*-aminobenzamides that are potent inhibitors of Hsp90 in an enzyme and cell-proliferation assay. In this Letter, we wish to disclose a series of macrocycles which show good to excellent solubility and microsomal stability.

The synthesis of our macrocyclic target structures commenced with allyl tetrahydroindolone **6** or the corresponding homoallyl derivative **7** (Scheme 1). Dihydroxylation and cleavage[21] of diols **8** or **9** yielded aldehydes **10** or **11**, respectively. Reductive amination of these aldehydes with a variety of mono-Boc protected[22] diamines **12** provided amines **13** following the removal of the protecting group with TFA. The intramolecular cyclization was accomplished in moderate to very good yields using Buchwald–Hartwig conditions for the arylation of amines[23] which was followed by hydrolysis of the benzonitriles to the benzamides **14**–**23** under either basic[19] or acidic conditions. While the synthesis of macrocyclic rings using palladium-mediated chemistry has been demonstrated to some extend before[24,25], this present work represents to the best of our knowledge the first application of the Buchwald–Hartwig reaction to access complex macrocyclic anilines.

Several 11- to 13-membered macrocycles were prepared using the approach illustrated in Scheme 1. The synthesis of a 14-membered macrocycle is outlined in Scheme 2. To this end, aldehyde **10** was reduced to the alcohol with $NaBH_4$ followed by conversion to the corresponding mesylate which was displaced with excess bis(2-aminoethyl)ether to produce diamine **24**. The macrocyclization was carried out with moderate yield using the same Pd-mediated conditions as before followed by the hydrolysis of the benzonitrile under basic conditions providing the ether linked macrocycle **25**.

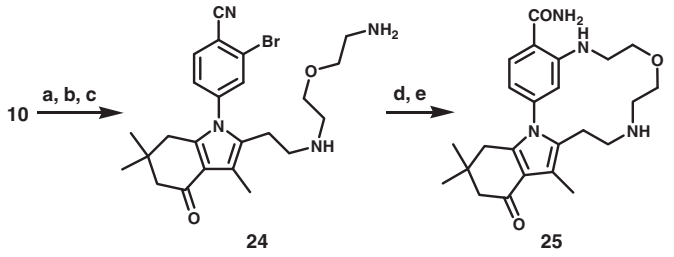
The synthesis of two structurally related 12-membered analogs is highlighted in Scheme 3. For this purpose the allyl side-chain in **26** was isomerized to the methylvinyl substituent in **27** by treatment with Grubbs 2nd generation catalyst in methanol at elevated temperature.[26] Following the arylation of pyrrole **28**, the obtained olefine **28** was dihydroxylated as before providing diol **29** which was converted to aldehyde **30** by treatment with sodium periodate. The target compounds **33** and **34** were prepared by reductive amination of aldehyde **30** using the mono-protected 1,4-diamino butane building blocks **31** or **32**, deprotection, Buchwald–Hartwig cyclization and hydrolysis.

To access another set of 12-membered amine-containing macrocycles, homoallyl derivative **7** was successfully subjected to Wacker oxidation conditions providing the corresponding methyl



#	m	n	X	Y	R	R'
14	1	1	H	H	H	H
15	1	0	H	H	H	H
16	2	1	Me	Me	H	H
17	2	0	Me	Me	H	H
18	2	0	H	H	H	H
19	2	0	H	H	Me	H
20	1	1	H	H	Me	H
21	2	0	H	H	Me	Me
22	1	1	Me	H	H	H
23	1	1	OH	H	H	H

Scheme 1. Reagents and condition: (a) OsO_4, NMO, *t*BuOH, 70–80%; (b) $NaIO_4/SiO_2$, DCM, quant.; (c) $NaBH(OAc)_3$, DCE, 14–95%; (d) 10% TFA, DCM, quant.; (e) Pd_2dba_3, BINAP, NaO*t*Bu, dioxane, toluene, 110 °C, 15–93%; (f) 90% H_2SO_4, 60 °C 19–48% or 5 M NaOH, 30% H_2O_2, DMSO, EtOH, 21–99%.



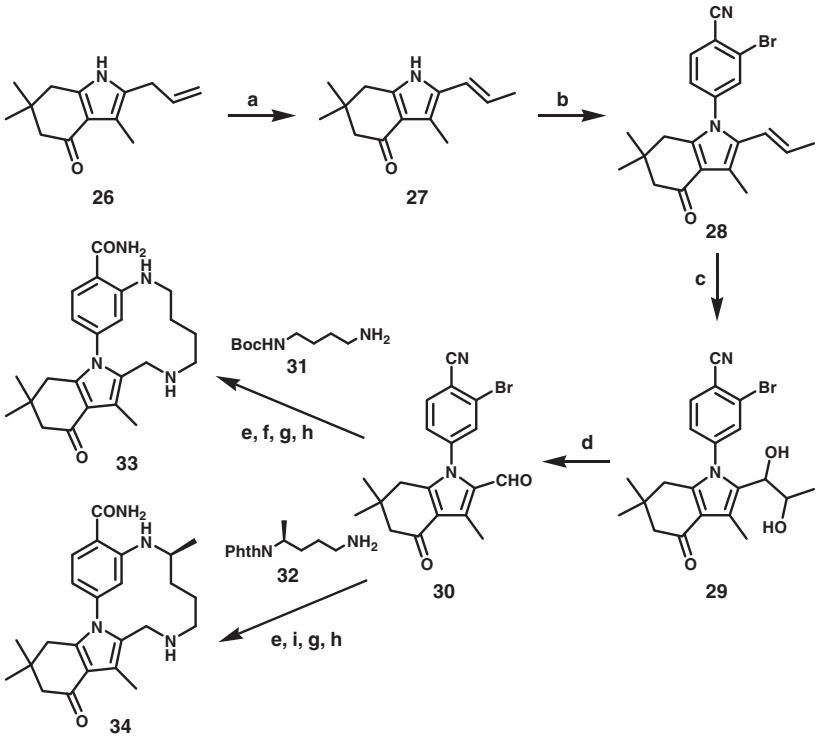
Scheme 2. Reagents and condition: (a) $NaBH_4$, THF, 0 °C, 71%; (b) $MeSO_2Cl$, Et_3N, DCM; (c) bis(2-aminoethyl)ether, 40 °C, 64%; (d) Pd_2dba_3, BINAP, NaO*t*Bu, toluene, 110 °C, 69%; (e) 5 M NaOH, 30% H_2O_2, DMSO, EtOH, quant.

ketone which after reductive amination with Boc-protected ethylene diamines **35a** (R = H) or 35b (R = Me) gave rise to racemic diamine **36a** or **36b** as a 1:1 mixture of diastereomers (Scheme 4). Both intermediates were deprotected and cyclized as before. The diastereomeric mixture of macrocycles obtained after the cyclization of deprotected diamine **36b** was separated at this stage by chromatography. Hydrolysis of the benzonitriles provided the three macrocyclic target structures **37–39**.

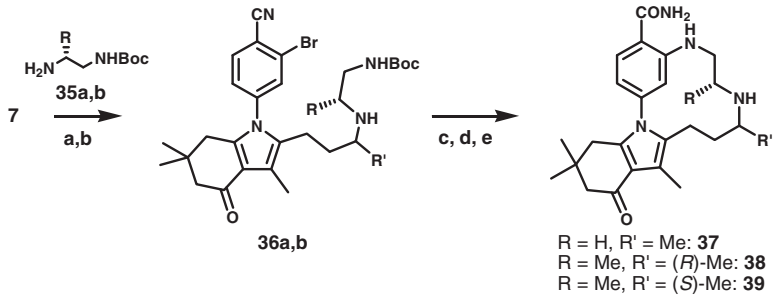
Further derivatization of macrocycles **19** and **20** by alkylation with iodomethane and acetic anhydride, respectively, yielded the corresponding tertiary amine **40** and acetamide **41** (Scheme 5).

Correlating the biologic activity of these macrocycles with their ring size was an important question at the outset of this project that needed to be addressed. This class of compounds forms two critical hydrogen bonds with Hsp90: the benzamide interacts with Asp93 while the carbonyl on the tetrahydroindolone moiety engages Tyr139 (see Fig. 2). Simple molecular modeling considerations suggest an optimal dihedral angle range for the aromatic moieties allowing these two hydrogen bonds to be accessible. It was expected that the tether length would influence this dihedral angle and thus the biologic activity of the macrocycle.

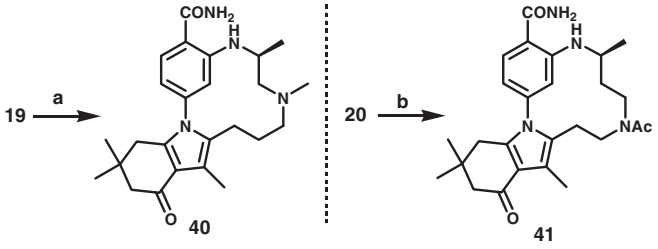
Target compounds were prepared in which the length of the tether had been varied. A comparison reveals that the 11-membered ring **15** is much less active than the corresponding 12-membered analog **14** (Table 1). While 13-membered analog **16** is more



Scheme 3. Reagents and condition: (a) Grubbs 2nd generation catalyst, MeOH, 60 °C, 70%; (b) NaH, 2-bromo-4-fluorobenzonitrile, DMF, 80 °C, 70%; (c) OsO_4, NMO, dioxane 61%; (d) $NaIO_4$, DCM, 82%; (e) $NaBH(OAc)_3$, DCE, **31**: 67%, 32: 23%; (f) 10% TFA, DCM, quant.; (g) Pd_2dba_3, BINAP, NaO*t*Bu, dioxane, toluene, 110 °C, for **33**: 11%, for **34**: 50%; (h) 5 M NaOH, 30% H_2O_2, DMSO, EtOH, **33**: 84%, **34**: 69%; (i) hydrazine, THF, 63%.



Scheme 4. Reagents and condition: (a) $PdCl_2$, CuCl, O_2, DMF, H_2O 78%; (b) **35a** or **35b**, $NaBH(OAc)_3$, DCE; R = H: 79%, R = Me: 83%; (c) 10% TFA, DCM, quant.; (d) Pd_2dba_3, BINAP, NaO*t*Bu, dioxane, toluene, 110 °C, for **36a**: 52%, for **36b**: 50% (~1:1 mixture of separable diastereomers); (e) 5 M NaOH, 30% H_2O_2, DMSO, EtOH, **37**: 36%, **38**: 69%, **39**: 78%.



Scheme 5. (a) MeI, Hünig's base, DCM, 27%; (b) Ac_2O, Et_3N, DCM, 85%.

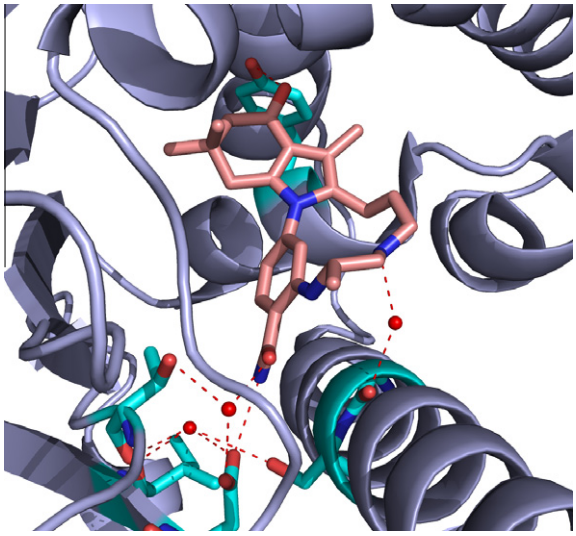


Figure 2. X-ray crystal structure of macrocycle **19** (pink) bound to the *N*-terminal ATP-binding site of Hsp90. Water mediated hydrogen bonds to the protein are highlighted in red. Selected water molecules are displayed as red spheres.

active than **14** in binding and proliferation activity, we also recognized that the gem-dimethyl substituents within the ring could be responsible for a favorable reduction in flexibility of the tether. The analogous 12-membered analog **17** also containing a gem-dimethyl unit was prepared leading to an increase in binding and cell-proliferation activity thus substantiating the need for a rigidifying element within the tether. This finding is underscored when comparing compound **17** with **18** which lacks the gem-dimethyl group and shows reduced biologic activity. Increasing the ring size from a 13- (**16**) to a 14-membered ring (**25**) leads to a further decrease in biologic activity. Together, these results led us to investigate 12-membered macrocycles containing substituents within the tether that contribute to a reduction of the compound's overall flexibility.

In a related series of analogs[27] we discovered the benefit of a properly configured chiral methyl group adjacent to the aniline nitrogen atom. To evaluate the effect of this substituent in the heteroatom-linked series of macrocycles, analogs **19** and **20** were prepared. As can be seen in Table 1, **17** and **19** are about equipotent in the binding and cell-proliferation assays underscoring that this chiral substituent provides the same effect on the biologic activity as the gem-dimethyl unit. Removing the chirality in the macrocycle by the addition of a methyl group (**21**) reduces the biologic activity. This finding confirms the stereochemical preference for substituents at this position of the ring.

Macrocycles featuring tethers with only one substituent are represented by racemic compounds **22** (X = Me) and **23** (X = OH). These compounds are less active when compared to the gem-dimethyl compounds **16** and **17**, presumably due to a lack of sufficient rigidity in the linker.

Table 1
Biologic activities, profiling data, and selected calculated properties for Geldanamycin and macrocycles **14–41**

Compd	Ring size	Hsp90[a]	HCT116[b]	Sol.[c]	Stab.[d]	MW[e]	*c* log *P*[f]
1[g]	19	0.04	0.03	NT	NT	561	2.1
14	12	0.40	8.14	NT	NT	395	3.3
15	11	12.44	NT	>100	15	380	3.1
16	13	0.29	0.21	6	3	437	4.7
17	12	0.14	0.08	52	15	423	4.4
18	12	0.92	0.82	35	>30	395	3.6
19	12	0.11	0.09	>100	>30	409	4.1
20	12	0.21	0.16	67	14	409	3.8
21	12	0.83	0.62	45	>30	423	4.6
22	12	0.36	0.25	39	5	409	3.8
23	12	0.32	0.60	70	10	411	2.6
25	14	0.57	0.83	73	28	425	2.9
33	12	>5.00	>5.00	18	18	395	3.3
34	12	>5.00	>5.00	16	7	409	3.8
37	12	1.24	0.94	69	26	409	4.1
38	12	0.09	0.03	>100	15	423	4.6
39	12	0.43	0.36	58	7	423	4.6
40	12	0.15	0.08	31	11	423	4.4
41	12	0.11	0.15	19	9	451	2.8

For experimental details see Ref. 20.
[a] Enzyme IC_{50} (μM).
[b] Cell-proliferation EC_{50} (μM).
[c] Solubility (μg/mL).
[d] $t_{1/2}$ in rat microsomes (min).
[e] Molecular weight.
[f] Calculated partition ratio.
[g] In-house data.

With respect to optimizing the position of the heteroatom within the tether, compound **19** is about twice as potent as **20** in binding and proliferation. Shifting the position of the nitrogen another position in the tether, however, leads to a complete loss of activity of the target structure irrespective whether the chiral methyl group is absent (**33**) or present (**34**).

Analog **38** which contains two chiral methyl substituents shows a three-fold improvement in cell-proliferation potency compared to compound **19**. A comparison of **38** with **37** and **39** implies that the number and stereochemical nature of the substituents within the tether can have a dramatic effect on the biologic activity of the macrocycles. Lastly, modifications of the basic nitrogen in the macrocyclic tether that result in an altering of its hydrogen-bonding properties (**40**) or its basicity (**41**) is possible without significant change in potency.

Table 1 also highlights the great variability in solubility that was determined for these macrocycles which ranged from poor to excellent. Likewise but without much correlation, wide ranging rat microsomal stability was measured. Fortunately, compound **19**, which is among this series' most active compounds in the binding (IC_{50} = 0.11 μM) and proliferation assay (IC_{50} = 0.09 μM), shows high aqueous solubility (>100 μg/mL) and is stable in rat microsomes ($t_{1/2}$ > 30 min). Similarly, analog **38** is also highly potent (binding IC_{50} = 0.09 μM, proliferation IC_{50} = 0.03 μM) and soluble (>100 μg/mL), however its rat microsomal stability is moderate ($t_{1/2}$ = 15 min).

An X-ray crystal structure for macrocycle **19** bound to the *N*-terminal ATP-binding site of Hsp90 was obtained.[28] As can be seen in Figure 2, the carbonyl of the tetrahydroindolone engages in a hydrogen bond with Tyr139 while the benzamide interacts with Asp93 in a direct and a water-mediated hydrogen bond. The nitrogen of the tether forms an additional water-mediated interaction to the main chain carbonyl of Asn51 (the side-chain is omitted in Fig. 2). Figure 2 shows a second water molecule proximal to the benzamide interacting with Ser52, Ile91, Asp93 and Thr184

representing the dense network of conserved water molecules characteristic for the *N*-terminal ATP-binding site of Hsp90. Additional water molecules were observed in the crystal structure but were omitted in Figure 2 for clarity.

The X-ray structure of **19** confirms that the macrocyclic tether linking the aminobenzamide to the tetrahydroindolone is indeed oriented toward the solvent exposed region of the binding site. This will allow further functionalization and derivatization of the tether enabling the fine-tuning of biologic and physical properties. Improved analogs of these novel macrocycles which display excellent biomarker activity will be reported in due course.[27]

Acknowledgments

We are deeply indebted to our dedicated co-workers in the Discovery Analytic Chemistry Group in Pearl River especially Mark Tischler, Joe Ashcroft, Hui Tong, Mairead Young, Keiko Tabai, Elwira Muszynska. Continued managerial support of this work came from Tarek Mansour, Will Somers, Kim Arndt and Robert Abraham, who are also gratefully acknowledged.

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27. manuscript in preparation
28. The structure has been assigned PDB ID code 3QTF.