ELSEVIER

# monitor

## MOLECULES

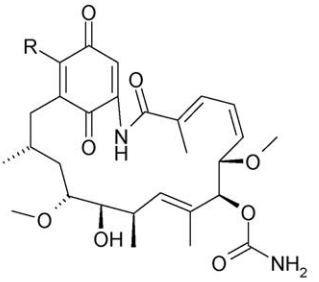
### The design and synthesis of focused parallel chemistry libraries for the discovery of biologically active substances

#### Dihydroxylphenyl amides as inhibitors of the Hsp90 molecular chaperone

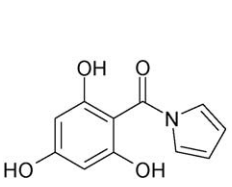
Molecular chaperones are protein machines that are responsible for the correct folding, stabilization, and function of other proteins in the cell [1]. Among the multitude of molecular chaperones and co-charperones identified to date is heat shock protein 90 (Hsp90), one of the most studied of this class of proteins. Hsp90 is a 90 kDa protein, is ATP-dependent and has multiple client proteins involved in the development and progression of cancer [2]. The client proteins rely on Hsp90 for their proper folding. In this regard, Hsp90 has been the focus of interest as a potential anticancer drug target. A number of natural product and semi-synthetic analogs have entered clinical trials as Hsp90 inhibitors, for example geldanamycin (i). There are issues, however, with compounds that have entered clinical trials to date. These compounds have potential therapeutic limitations because of low solubility, liver toxicity, and extensive metabolism [3]. In an effort to obviate these problems, recent work [4] has disclosed research into the identification of non-quinone-containing small molecule inhibitors of Hsp90 chaperone activity. This new research commenced with a high throughput screening campaign using a competitive binding assay, in which the ability of test compounds under study to displace tritium-labeled 17-propylamino-benzoquinone ansamycin (ii) from Hsp90 was assessed. The first compound of interest to arise out of this work was (iii) which, although potent in the competitive binding assay (Ki 200 nM), was unfortunately not potent ($IC_{50} > 20$ μM) in a cell-based assay measuring the degradation of the Hsp90 client protein, Akt. To move the project forward, these researchers obtained a 1.4 Å co-crystal structure of compound (iii) bound to Hsp90 and compared it to the crystal structures of other known Hsp90 inhibitors complexed with the protein. This led to the discovery that only two of the three hydroxyl groups on (iii) make key hydrogen bonding interactions with conserved water molecules. Thus, there existed the possibility of replacing one hydroxyl moiety with other groups that could modulate not only cell-based potency, but also physicochemical properties in the designed molecules. To this end, the researchers designed a small library in which commercially available amines were used to replace the pyrrole moiety present in inhibitor (iii), as well as dispense with one hydroxyl group in favor of a hydrogen atom. After synthesis and biological screening, it was found that generally library compounds derived from primary amines were less potent Hsp90 inhibitors than those prepared from secondary amines. One of the most potent compounds isolated at this stage of the research program was (iv) that possessed a cell-based potency of 9.3 μM. In order to improve inhibitor potency further, compound (iv) was resolved by preparative chiral SFC (supercritical fluid chromatography) to obtain the corresponding single enantiomers. Of these, compound (v) was the most potent and displayed a Ki of 30 nM. Further medicinal chemistry design based around analysis of X-ray co-crystals coupled with synthesis enabled by parallel chemistry led to the discovery of compound (vi) that possessed a Ki of $<10$ nM and a cell-based potency of 20 nM. Thus, a series of potent Hsp90 inhibitors was discovered through the use of both focused library synthesis and structure-based drug design. Further work in this area is warranted to both broaden the SAR and understand the physicochemical properties in this series, and ultimately progress toward the creation of compounds with drug-like properties commensurate with oral delivery, and with no toxicity.



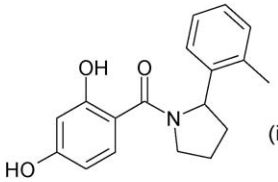
1359-6446/06/$ - see front matter  doi:10.1016/j.drudis.2009.05.001



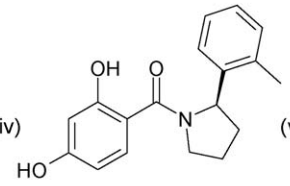
R = OMe; (i)
R = $NHCH_2CH_2CH_3$(ii)



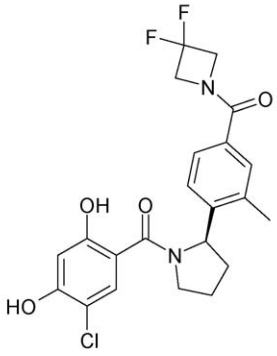
(iii)



(iv)



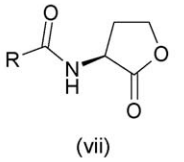
(v)



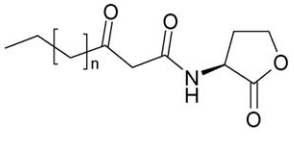
(vi)

## Evaluation of *N*-aryl L-homoserine lactones reveals a new set of quorum sensing modulators

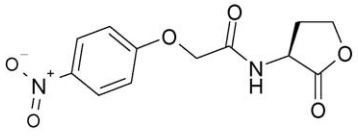
Bacteria can often assemble into multicellular communities and initiate processes as a group that, as individual cells, they are incapable of doing [5]. These group behaviors are under the control of a cell–cell signaling pathway called quorum sensing, and this plays a significant role in the establishment of both symbiotic and pathogenic relationships with eukaryotic hosts [6]. Processes under the control of quorum sensing are virulence factor production and biofilm formation. Quorum sensing is mediated by autoinducers and their cognate protein receptors; in other words, through signals produced by low molecular weight compounds. Autoinducer–receptor binding occurs once bacteria reach a threshold cell density and this binding event controls the transcription of genes necessary for bacterial group functions. Interception of this binding event represents a strategy to attenuate bacterial group behaviors, and has thus been considered as an approach to the control of bacteria. Recent work [7] disclosed the creation of a set of autoinducer mimics that are derived from earlier disclosed compounds of general structure (vii). Subtle structural changes in the 'R' group in structure (vii) have been shown to have a marked effect on ligand activity [8]. In designing and synthesizing a solution phase, microwave-assisted, library of derivatives of (vii), various spacers and heteroatoms were incorporated within the 'R' group when referenced to the core, in order to generate a wide diversity of substituents and thus SAR. The compounds from this library were evaluated for antagonistic and agonistic activity in LuxR-type receptors in *A. tumefaciens*, *P. aeruginosa* and *V. fischer*i (*TraR*, *LasR*, and *LuxR*, respectively), using cell-based reporter gene assays according to reported procedures [8]. *LuxR* activation or inhibition in the *V. fischeri* strain was reported by luciferase production. Antagonism assays were performed in the presence of library compound and native *N*-acylated L-homoserine lactone ligand (viii–x) at its approximate $EC_{50}$ value, while agonism assays were performed with library compound alone. The native *N*-acylated L-homoserine lactone ligands for *A. tumefaciens* (ix), *P. aeruginosa* (x), and *V. fischeri* (viii) were used as controls for these assays. On the basis of these assays, a number of active compounds were obtained. One of the most potent compounds obtained was (xi) which possessed $IC_{50}$ values of 0.44 μM, 2 μM and 1.9 μM against *A. tumefaciens TraR*, *E. coli LasR* and *V. fischeri LuxR*, respectively. This work is of interest because the disclosed library work has delivered a new set of synthetic LuxR-type receptor antagonists and agonists, thus serving to underscore the utility of screening focused *N*-acylated L-homoserine lactone ligand libraries for the optimization of existing, and the identification of new, LuxR-type receptor modulators. Further work in this area is warranted to progress our level of understanding of the biological activity with these series of compounds, and thus deliver compounds suitable for clinical evaluation.



(vii)



n = 1: viii
n = 3: ix
n = 7: x



(xi)

1 Jolly, C. and Morimoto, R.I. (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J. Natl. Cancer Inst.* 92, 1564–1572
2 Maloney, A. and Workman, P. (2002) HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin. Biol. Ther.* 2, 3–24
3 Workman, P. (2004) Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett.* 206, 149–157
4 Pei-Pei Kung, *et al.* (2008) Dihydroxylphenyl amides as inhibitors of the Hsp90 molecular chaperone. *Bioorg. Med. Chem. Lett.* 18, 6273–6278
5 Camilli, A. and Bassler, B.L. (2006) Bacterial small-molecule signaling pathways. *Science* 311, 1113–1116
6 Greenberg, E.P. (2003) Bacterial communication: tiny teamwork. *Nature* 424, 134
7 Geske, G.D. *et al.* (2008) Evaluation of a focused library of *N*-aryl L-homoserine lactones reveals a new set of potent quorum sensing modulators. *Bioorg. Med. Chem. Lett.* 18, 5978–5981
8 Geske, G.D. *et al.* (2007) Modulation of bacterial quorum sensing with synthetic ligands: systematic evaluation of *N*-acylated homoserine lactones in multiple species and new insights into their mechanisms of action. *J. Am. Chem. Soc.* 129, 13613–13625

***Paul Edwards***
*mepauledwards@fsmail.net*