European Journal of Medicinal Chemistry 143 (2018) 390–401

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design, synthesis, and biological evaluation of a series of resorcinol-based *N*-benzyl benzamide derivatives as potent Hsp90 inhibitors

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ARTICLE INFO

Article history:
Received 5 September 2017
Received in revised form
19 October 2017
Accepted 20 November 2017
Available online 21 November 2017

Keywords:
Hsp90 inhibitor
Lung cancer
Resistance
Small molecule
Drug design

ABSTRACT

Heat shock protein 90 (Hsp90) is a ubiquitous molecular chaperone that is responsible for the stabilization and maturation of many oncogenic proteins. Therefore, Hsp90 has emerged as an attractive target in the field of cancer chemotherapy. In this study, we report the design, synthesis, and biological evaluation of a series of Hsp90 inhibitors. In particular, compound **30f** shows a significant Hsp90α inhibitory activity with IC_{50} value of 5.3 nM and an excellent growth inhibition with GI_{50} value of 0.42 μM against non-small cell lung cancer cells, H1975. Compound **30f** effectively reduces the expression levels of Hsp90 client proteins including Her2, EGFR, Met, Akt, and c-Raf. Consequently, compound **30f** promotes substantial cleavages of PARP, Caspase 3, and Caspase 8, indicating that **30f** induces cancer cell death via apoptotic pathway. Moreover, cytochrome P450 assay indicates that compound **30f** has weak inhibitory effect on the activities of five major P450 isoforms (IC_{50} > 5 μM for 1A2, 2C9, 2C19, 2D6, and 3A), suggesting that clinical interactions between **30f** and the substrate drugs of the five major P450 isoforms are not expected. Compound **30f** also inhibits the tumor growth in a mouse xenograft model bearing subcutaneous H1975 without noticeable abnormal behavior and body weight changes. The immunostaining and western immunoblot analysis of EGFR, Met, Akt in xenograft tissue sections of tumor further demonstrate a good agreement with the *in vitro* results.

1. Introduction

The molecular chaperone heat shock protein 90 (Hsp90) maintains protein homeostasis under numerous stressors by regulating the correct folding, stability, and activity of various client proteins [1–4]. Hsp90 clients include many oncogenic proteins, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), mesenchymal-epithelial transition tyrosine receptor (Met), anaplastic lymphoma kinase (Alk), protein kinase B (Akt/PKB), cellular rapidly accelerated fibrosarcoma (c-Raf), cyclin-dependent kinase 4 (Cdk4), hypoxia-inducible factor 1α (Hif-1α), matrix metalloproteinase 2 (MMP2), mutant P53 and wee1, which are necessary for the development and progression of cancer. Due to the hostile environment in cancers such as hypoxia, acidosis, and nutrition deprivation, Hsp90 is overexpressed in cancer cells 2–10 fold higher than normal cells and exists as activated multi-chaperone complexes in cancer cells [5]. In this regard, cancer cells are highly addicted to Hsp90 chaperone function for the survival and proliferation. More importantly, inhibition of Hsp90 results in simultaneous blockage of multiple signaling pathways in cancers, overcoming the inevitable drug resistance of conventional chemotherapies. Therefore, inhibition of the molecular chaperone Hsp90 represents a promising chemotherapeutic strategy toward the treatment of various types of cancers.

Over the past decades, a substantial number of Hsp90 inhibitors have been developed [6–13]. The ansamycin antibiotic, geldanamycin (**1**) was first identified as an Hsp90 inhibitor in 1994 (Fig. 1) [14]. Since then, a number of geldanamycin analogues such as

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https://doi.org/10.1016/j.ejmech.2017.11.054
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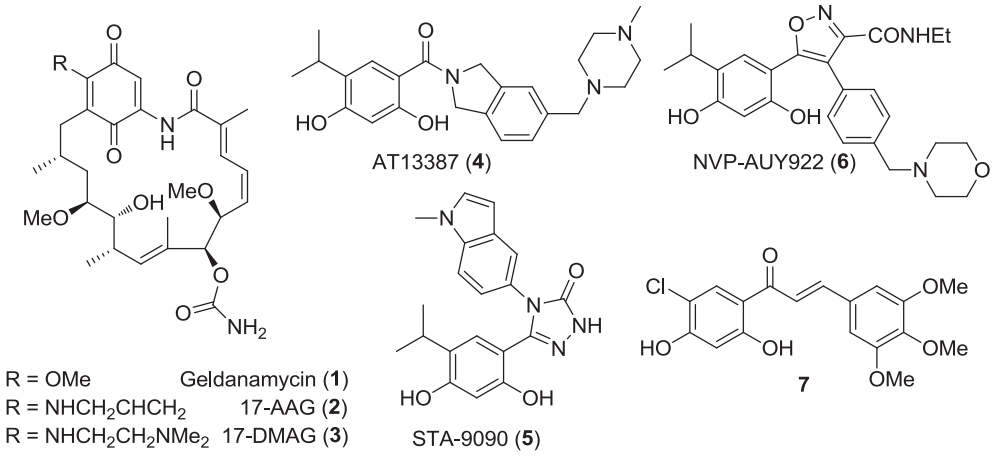


Fig. 1. Structure of Hsp90 inhibitors.

tanespimycin (17-AAG, **2**) and alvespimycin (17-DMAG, **3**) have been developed and entered into clinical studies [8]. However, the first-generation ansamycin derivatives engendered several drawbacks in the clinical applications including poor solubility and toxicity [15]. Consequently, substantial efforts had been directed towards finding different chemical scaffolds to improve pharmacological properties as well as safety profiles, resulting in the second-generation Hsp90 inhibitors. These second-generation Hsp90 inhibitors are classified into three major cores, purine, resorcinol, and benzamide. The purine class of Hsp90 inhibitors that mimics the adenine ring of natural nucleotide ligand ATP, includes PU-H71 [16], BIIB021 [17], and CUDC-305 [18] and resorcinol-based Hsp90 inhibitors include AT13387 (**4**) [19], STA-9090 (**5**) [20], NVP-AUY922 (**6**) [21]. The benzamide scaffold is another important class of Hsp90 inhibitors, which includes TAS-116 [22], XL888 [23] and SNX-5422 [24]. A number of the second-generation Hsp90 inhibitors have entered to the clinical trials and demonstrate an improved potency with lesser toxicities [6,25,26]. However, none of the Hsp90 inhibitors are clinically approved yet. Most recently, Hsp90 inhibitor STA-9090 has failed to demonstrate the clinical benefit in the phase III clinical trial due to the moderate efficacy and consequently the clinical trial has been terminated [26]. Although the clear reasons for the failure are still under investigation, acquired resistance to STA-9090 has been proposed as one possible reason [27]. In this regard, the discovery of Hsp90 inhibitors with different chemotypes is still a demanding task in this area.

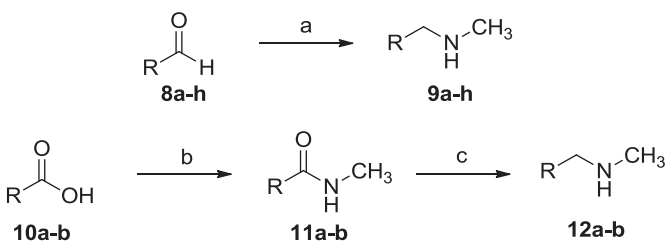
As part of our ongoing effort to develop potent Hsp90 inhibitors, we have previously discovered a chalcone-based Hsp90 inhibitor (**7**) by utilizing a fragment-linking strategy [28–31]. Despite its potential as an anticancer drug, there are several challenges for its clinical application. One is that, owing to the intrinsic electrophilic characteristic of the enone moiety that acts as Michael acceptor, chalcone structure of compound **7** might exert a non-specific binding issue and render compound **7** susceptible to be attacked by endogenous cellular nucleophiles such as cysteine, lysine, and histidine [32,33]. Besides, there still remains a need for the improvement of the potency. Therefore, we decided to replace the enone moiety with various amide groups, ruling out the possibility of its non-specific binding events.

2. Results and discussion

2.1. Chemistry

Our synthesis began with preparation of benzyl amines **9a-h**. Reductive amination of benzaldehydes **8a-h** with methyl amine and sodium borohydride gave benzyl amines **9a-h** in 22–100% yields (Scheme 1). Alternatively, benzoic acids **10a-b** was converted to amides **11a-b** in 11–41% yields using EDC, HOBt and DIPEA in DMF, which was then reduced using lithium aluminum hydride to furnish benzyl amines **12a-b** in 65–81% yields.

We next synthesized compound **20** and **21a-h**, which had chloro substituent on resorcinol ring (Scheme 2). The synthesis of **20** and **21a-h** commenced with esterification of commercially available **13**, providing ester **14** in 86% yield. Subsequent chlorination of **14** with sulfuryl chloride in dichloromethane gave **15** in 45% yield. Compound **15** was then protected with allyl bromide and potassium carbonate in DMF, quantitatively to furnish compound **16**. Compound **16** was hydrolyzed with sodium hydroxide in methanol and water to give carboxylic acid **17** in 80% yield. With carboxylic acid **17** and amine **9a-h** in hand, we carried out amide coupling reactions of **17** with **9a-h** or benzyl amine using EDC, HOBt and DIPEA in DMF, followed by cleavage of allyl-protecting group using $PdCl_2(PPh_3)_2$ reaction, consequently to provide compound **20** and **21a-h** in 21–90% yields in two steps. Although the synthetic route in Scheme 2 successfully provided compound **20** and **21a-h**, it required a lengthy synthesis with an additional protection and deprotection reaction steps. Besides, the yield of allyl cleavage reaction was unexpectedly low. Hence, we examined the viability of direct amide coupling reaction of **22** without protecting group. To do so, we first hydrolyzed the ester **15** with sodium hydroxide in methanol and water to obtain carboxylic acid **22** in quantitative yield. With **22** in hand, we carried out amide coupling reaction of **22** with amine **12a-b**. To our delight, the reaction using EDC, HOBt and DIPEA in DMF at 120 °C under microwave irradiation afforded compound **23a-b** in 69% and 26% yield.



Scheme 1. Synthesis of compound **9a-h** and **12a-b**[a]
[a]Reagents and conditions: (a) CH_3NH_2, $NaBH_4$, MeOH, 0 °C, 1.5 h; (b) CH_3NH_2, EDC, HOBt, DIPEA, DMF, microwave, 120 °C, 30 bar, 3 h; (c) $LiAlH_4$, THF, 0 °C, 12 h.