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Original article

Isoxazolo(aza)naphthoquinones: A new class of cytotoxic Hsp90 inhibitors

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ABSTRACT

A series of 3-aryl-naphtho[2,3–*d*]isoxazole-4,9-diones and some of their 6-aza analogues were synthesized and found to inhibit the heat shock protein 90 (Hsp90). The compounds were tested for their binding to Hsp90 and for their effects on Hsp90 client proteins expression in a series of human tumour cell lines. Representative compounds (**7f**, **10c**) downregulated the Hsp90 client proteins EGFR, Akt, Cdk4, Raf-1, and survivin, and upregulated Hsp70. Most of the compounds, in particular the alkylated 3-pyridyl derivatives, exhibited potent antiproliferative activity, down to two-digit nanomolar range. Preliminary results indicated *in vivo* activity of **7f** against human epithelial carcinoma A431 model growing as tumour xenograft in nude mice, thus supporting the therapeutic potential of this novel series of Hsp90 inhibitors.

1. Introduction

The interest for the heat shock protein 90 (Hsp90) as a therapeutic target is related to its central role in correct folding and stabilization of proteins involved in malignant behaviour and tumour progression [1]. Multiple signal transduction pathways implicated in the regulation of cell proliferation and survival are dependent on Hsp90 [2]. Several Hsp90 client proteins are involved in critical processes including cell-cycle regulation and apoptosis [3]. The heat shock proteins are often overexpressed in tumour cells, and this supports their ability to survive under unfavourable stress conditions (e.g. hypoxia and acidosis). The essential chaperoning function of Hsp90 is subverted during oncogenesis to make malignant transformation possible and to facilitate rapid somatic evolution [3]. Functioning as a biochemical buffer for the numerous genetic lesions that are present within tumours, Hsp90 allows mutant proteins to retain or even gain function, while permitting cancer cells to tolerate the imbalanced signalling that such oncoproteins create. Thus, targeting Hsp90 may have the potential advantage of simultaneously blocking multiple oncogenic pathways [4].

Hsp90 exists as a homodimer made up of three domains [1,2]. The N-terminal domain contains an ATP-binding site that binds the natural products geldanamycin and radicicol, and the analogue 17-AAG (Chart 1). The middle domain is highly charged and has high affinity for cochaperones and client proteins. A second ATP-binding site is located in the C-terminus of Hsp90. This C-terminal nucleotide binding pocket has been shown to bind not only ATP, but cisplatin, novobiocin, epigallocatechin-3-gallate (EGCG) and taxol [5].

A number of highly specific Hsp90 inhibitors have been identified [6]. They redirect Hsp90 chaperoning activity and decrease cellular levels of its numerous cancer-related client proteins [2]. Such inhibitors exhibit promising antitumour activity as single

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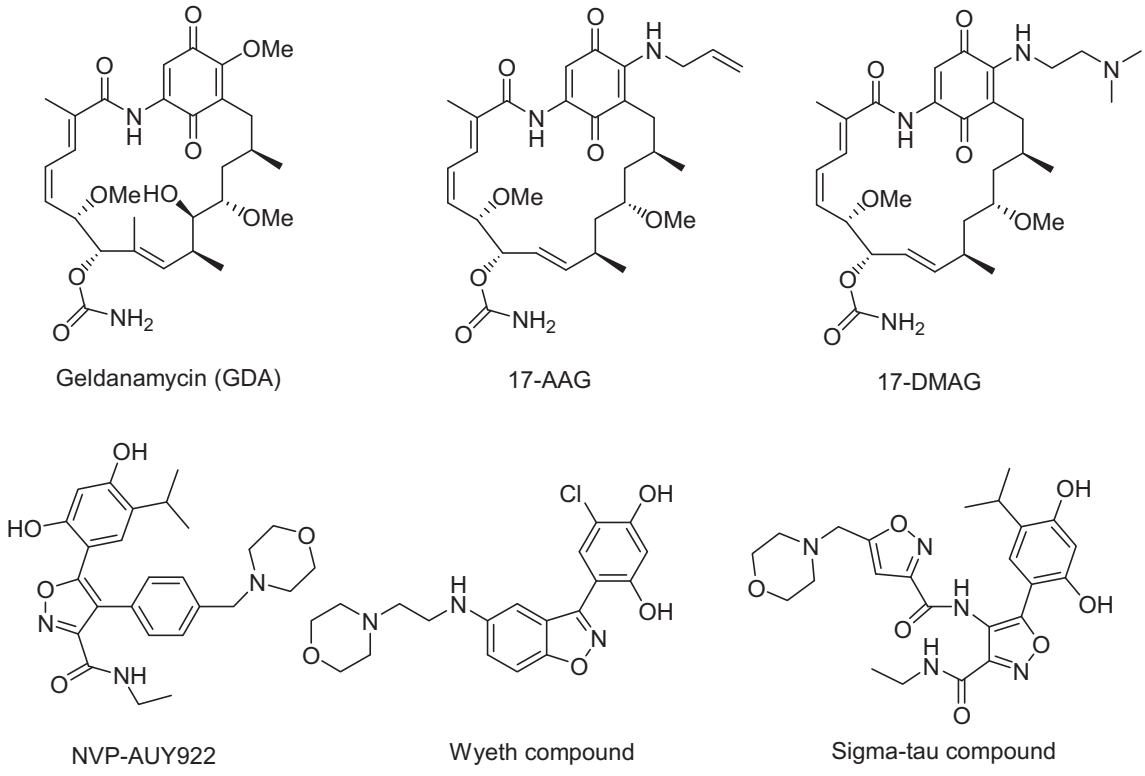


Chart 1.

agents or in combination with other cytotoxic agents [7–9], and some of them are in clinical development [10,11]. Geldanamycin, the first Hsp90 inhibitor discovered, and its derivatives 17-AAG (tanespimycin) and 17-DMAG (17-dimethylamino-ethylamino-17-demethoxygeldanamycin, alvespimycin), that have entered clinical trials, are ansa compounds containing a key aminoquinone moiety [12]. (Chart 1).

Quinones are a class of organic compounds endowed with a variety of biological activities, mostly connected with their redox properties. A number of natural and synthetic quinones show remarkable anticancer activity [12], and a series of synthetic 1,4-naphtoquinones have recently been identified as Hsp90 inhibitors [13].

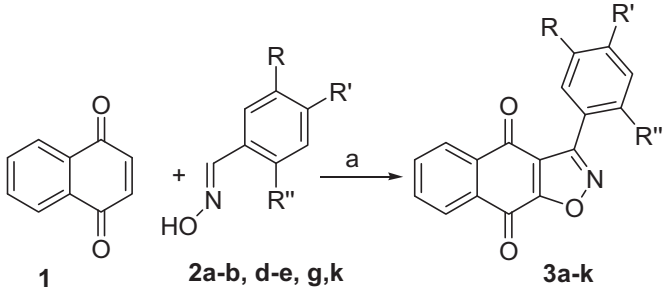
Moreover, among the large number of Hsp90 inhibitors, some compounds, containing the isoxazole nucleus (Chart 1), have shown potent and selective inhibition of this molecular chaperone [14]. The presence of the heterocyclic nucleus seems to exert a role in the docking of the compounds to the ATP-binding site of Hsp90 [15].

This paper reports the synthesis and the assessment of the cytotoxic activity of a series of new isoxazolo-fused naphthoquinones and isoquinolinoquinones, that exert their antitumour activity via inhibition of the molecular chaperone Hsp90.

2. Chemistry

The 1,3-dipolar cycloaddition of benzonitriloxides to quinones is a well–known reaction [16] that has been exploited for the synthesis of simple and substituted isoxazolo-fused quinones [17] and naphthoquinones [18]. Thus, the first series of compounds (**3a**–**p**) was prepared reacting the naphthoquinone **1** with the nitrile oxides obtained in situ by treating the corresponding oximes **2** with triethylamine and aqueous NaClO in dichloromethane [19] (Schemes 1 and 2).

Some compounds (**3c**,**f**,**h**,**i**) were prepared by demethylation of the corresponding ethers (**3b**,**e**,**g**). Compounds **6a**–**e** were obtained regioselectively [20] by 1,3-dipolar cycloaddition on the



Scheme 1. Reagents and conditions: a) TEA, 15% NaClO, CH_2Cl_2, 0 °C, 1.5 h; b) HBr 35%, AcOH, reflux, 5 h; c) BBr_3, CH_2Cl_2, 0 °C, 1 h, then rt, 30 min; d) BBr_3, CH_2Cl_2, 0 °C, 1.5 h, then rt, 1 h; e) HBr 35%, AcOH, reflux, 48 h.