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New TRAP1 and Hsp90 chaperone inhibitors with cationic components: Preliminary studies on mitochondrial targeting

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ABSTRACT

TRAP1 (Hsp75) is the mitochondrial paralog of the Hsp90 molecular chaperone family. Due to structural similarity among Hsp90 chaperones, a potential strategy to induce apoptosis through mitochondrial TRAP1 ATPase inhibition has been envisaged and a series of compounds has been developed by binding the simple pharmacophoric core of known Hsp90 inhibitors with various appendages bearing a permanent cationic head, or a basic group highly ionizable at physiologic pH. Cationic appendages were selected as vehicles to deliver drugs to mitochondria. Indeed, masses of new derivatives were evidenced to accumulate in the mitochondrial fraction from colon carcinoma cells and a compound in the series, with a guanidine appendage, demonstrated good activity in inhibiting recombinant TRAP1 ATPase and cell growth and in inducing apoptotic cell death in colon carcinoma cells.

TRAP1 (Tumor Necrosis Factor-Associated Protein 1, a mitochondrial paralog of Hsp90 chaperone family, also known as Hsp75) is a component of a mitochondrial pathway, selectively up-regulated in tumor cells and responsible for maintenance of mitochondrial integrity, thus favoring cell survival. Studies demonstrated that mitochondrial TRAP1, together with Hsp90, interacts with cyclophilin D (Cyp D), a regulator of permeability transition pore, and antagonizes the Cyp D-dependent apoptotic cascade, likely via a protein (re)folding mechanism.[1] In a context of cancer cells with TRAP1 overexpression, its silencing was demonstrated to cause sudden growth inhibition and apoptosis, and this correlated with altered mitochondrial function and modified protein expression, thus suggesting that this pathway may represent a novel molecular target for anticancer therapy.[2] In this perspective, an attractive idea has been recently proposed regarding the delivery of TRAP1 inhibitors inside mitochondria, as a tool to conjugate anticancer activity together with selectivity toward cancer cells with high mitochondrial TRAP1 levels.[3] TRAP1 is also involved in protein homeostasis through an extramitochondrial quality control pathway involving the proteasome regulatory particle TBP7, and this function is relevant for TRAP1 antiapoptotic role.[4–6] Thus, several mechanisms are involved in multifaceted roles of TRAP1 in adaptive processes of cancer cells.[7–10]

Several attempts have been made in recent years to increase the efficacy of anticancer therapy through a specific subcellular compartmentalization delivery of drugs. In this field, mitochondria have been considered an attractive target for their relevant metabolic roles altered in cancer models. An appropriate and specific drug-delivery system is required to design mitochondria-targeted drugs.[11–16] Indeed, many structures able to direct pharmacological compounds to mitochondria share the presence of a basic component or a permanent cationic lipophilic group, in order to cross over membranes by exploiting a very favorable electric gradient. For example, groups such as polyamines, protonated at physiological pH, have been successfully employed to carry and internalize biologically active compounds through mitochondrial membranes into the organelle.[17] These structures, indeed, not only allow mitochondrial membrane crossing, but also favor specific accumulation in the organelle. In the field of Hsp90 inhibitors, the so-called Gamitrinibs, a family of geldanamycin derivatives (17-AAG)

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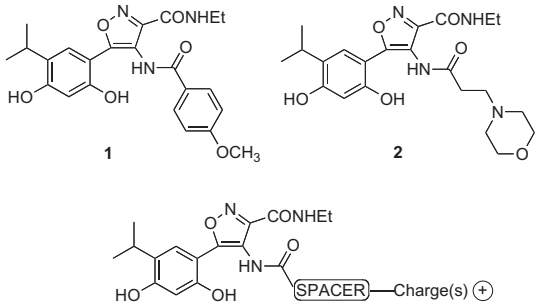


Fig. 1. Known Hsp90 inhibitors and general structure of potential mitochondrial targeting derivatives.

linked to cyclic guanidines or triphenylphosphonium groups, have been proposed as a novel class of mitochondria-directed TRAP1/Hsp90 inhibitors.[18,19,3] Our study took advantage from the availability of a TRAP1 crystal structure recently provided by Sung and co-workers.[20] In fact, a further issue, besides the specific mitochondrial delivery, deals with the selectivity of TRAP1/Hsp90 ATPase inhibition. To this aim we searched for novel putative TRAP1 antagonists and, in this study, a number of derivatives, as compounds **1** and **2** in Fig. 1, already known to be non- or partly ionizable Hsp90 inhibitors,[21] were selected since they were predicted to interfere also with TRAP1 activity, based on sequence homology between Hsp90 and TRAP1 structures. Furthermore, the Hsp90 inhibitors **1** and **2** were modified to enhance their accumulation into mitochondria.

The feasibility of the project was first investigated by preparing a small explorative sample of a three-portion structure so equipped:

1) A common 3,4 isoxazole diamide structure, as seen in Fig. 1, derived from known compounds active as Hsp90 inhibitors. The nitrogen at position 4 of isoxazole is particularly suitable to link a multitude of different appendages;
2) A cationic head, either as a permanent ion or as an ionizable group at physiologic pH;
3) A spacer between the portions above described, that can be chosen of various lengths.

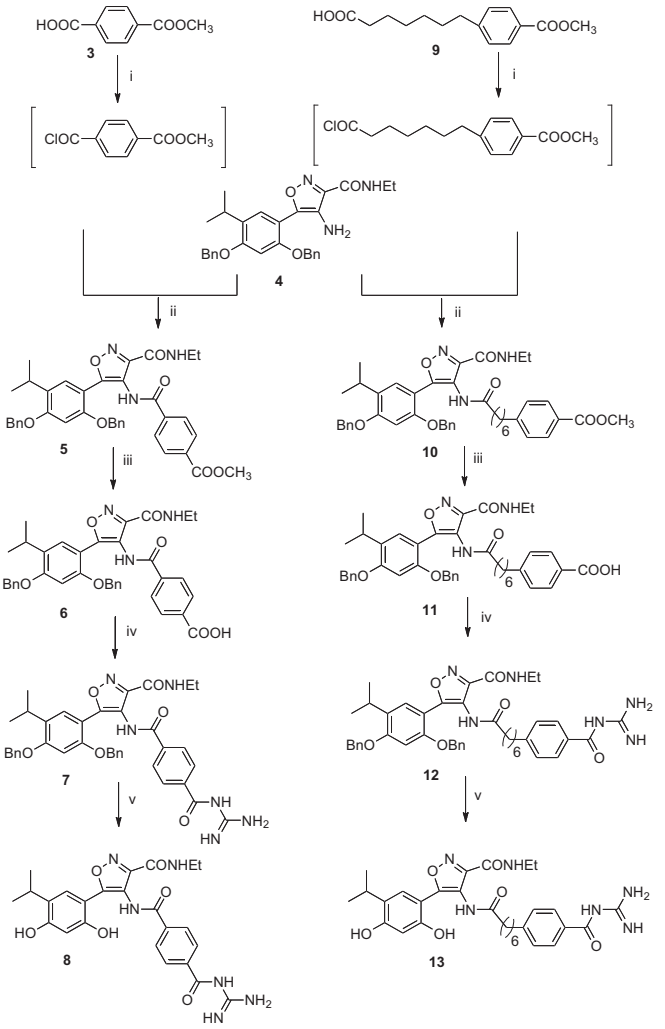
In particular, a series of triphenylphosphonium as well as of pyridinium salts, and a guanidinium or a polyamine appendage have been considered as cationic heads to be linked to a common intermediate (**4**, Scheme 1), and were obtained as previously reported.[21] Conjugate derivatives were comparatively evaluated by testing inhibition of recombinant TRAP1 ATPase activity, as well as accumulation in mitochondria, cell viability and induction of apoptosis in colorectal carcinoma cells.

The synthetic procedure is depicted in Schemes 1 and 2.

The guanidine vehicle was introduced using two linkers of different length: the terephthalic acid monomethyl ester and the 7-(4-methoxycarbonylphenyl)-heptanoic acid were converted into acyl chlorides and reacted with intermediate **4**, obtaining compounds **5** and **10**, respectively. Ester hydrolysis and coupling with guanidine (obtained from the hydrochloride treated with potassium tert-butoxide) gave intermediates **7** and **12**, finally deprotected with BCl_3 to yield compounds **8** and **13**.

Similarly, compound **4** was reacted with hexanedioic acid chloride monoethyl ester to obtain compound **15**, then hydrolized and coupled with the protected amine **17** (obtained as described in literature[22]). The deprotection of compound **18** gave the final polyamine derivative **19**.

Finally, the permanent cationic derivatives were synthesized reacting compound **4** with 6-bromohexanoic acid chloride, then deprotecting **21** with BCl_3 and displacing bromine of **22** with either pyridine (as refluxing solvent) or triphenylphosphine in refluxing 1,4-dioxane to give compounds **23** and **24**, respectively.



Scheme 1. Synthesis of guanidine bearing derivatives **8** and **13**. Reagents and conditions: (i): oxalyl chloride, DMF, CH_2Cl_2, 4 h, rt; (ii): CH_2Cl_2, TEA, 16 h, rt; (iii): MeOH, 1 N NaOH, H_2O, 24 h, 70 °C; (iv): DMF, CDI, 1 h, rt, CH_5N_3·HCl, 16 h, rt; (v): 1 M BCl_3, CH_2Cl_2, – 78 °C, 1 h.

All newly-synthetized compounds were tested for inhibition of recombinant TRAP1 ATPase activity and cell viability, accumulation in mitochondria, and induction of apoptosis in colon carcinoma HCT116 cells.

Based on the structural homology between TRAP1 and Hsp90, the newly synthesized compounds were tested in comparison with the isoxazole-amide **1**, recently emerged from our studies on Hsp90 inhibitors.[21] Two well-known potent Hsp90 inhibitors, i.e., AUY922 and Hsp990, obtained from Novartis, were also tested since they were also proposed as potentially acting on the TRAP1 ATPase domain.[23,24]

In preliminary experiments, the ability of the reported compounds to accumulate inside mitochondria was investigated. To this purpose, a mass spectrometric analysis on separated mitochondrial and cytosolic fractions purified from colorectal carcinoma HCT116 cells exposed to 1 μM of each agent for 12 h was used as a qualitative technology to assess the intracellular distribution of our compounds. We found molecular peaks corresponding to compounds **8**, **13**, **19** and **24,** but not to the reference compounds **1** and Hsp990, in the mitochondrial fractions of HCT116 cells. On the other hand, in the experimental conditions adopted, we did not find any of the tested compounds in the cytosolic compartment. Additional experiments are however necessary to quantitatively address the issue of the intracellular distribution of these compounds.