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Synergistic effect of pyrazoles derivatives and doxorubicin in claudin-low breast cancer subtype

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ABSTRACT

Background: Breast cancer is a global public health problem. For some subtypes, such as Claudin-low, the prognosis is poorer and the treatment is still a challenge. Pyrazoles are an important class of heterocyclic compounds and are promising anticancer agents based on their chemical properties. The present study was aimed not only at testing pyrazoles previously prepared by our research group in two breast cancer cell lines characterized by intermediated response to conventional chemotherapy but also at analyzing the possible synergistic effect of these pyrazoles associated with doxorubicin.

Methods: Four 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-1H pyrazoles were tested for the first time in MCF-7 and MDA-MB-231 culture cells. The pyrazoles with best results in cytotoxicity were used in combination with doxorubicin and compared with this drug alone as standard. The synergic effect was analyzed using Combination Index method. In addition, cell death and apoptosis assays were carried out.

Results: Two pyrazoles with cytotoxic effect in MCF-7 and especially in MDA-MB-231 were identified. This activity was markedly higher in pyrazoles containing bromine and chlorine substituents. The combination of these pyrazoles with doxorubicin had a significant synergic effect in both cells tested and mainly in MDA-MB-231. These data were confirmed with apoptosis and cell death analysis.

Conclusions: The synergic effect observed with combination of these pyrazoles and doxorubicin deserves special attention in Claudin-low breast cancer subtype. This should be explored in order to improve treatment results and minimize side effects.

1. Introduction

Breast cancer remains a disease of high prevalence and is the most frequently diagnosed cancer worldwide. In some countries, this is the leading cause of women cancer-related death [1,2]. This entity represents a heterogeneous disease, classified in distinct subsets by gene expression signature with important implications for treatment, responses and outcome. The choice of appropriate therapy and prognosis depends on a number of factors including the molecular subtype of the pathology, which is important for the identification of predictive factors for response to a given treatment [3–5].

Basal-like and Claudin-low subtypes, represent about 19% of all breast cancers including those with worst prognosis due to its aggressive and metastatic nature and high rates of relapse [6–8]. These are found into the bigger subset of triple negative tumors which lack the expression of estrogen, progesterone receptor (ER/PR) and do not exhibit amplification of the human epidermal growth factor receptor 2 (HER2) gene. MDA-MB 231 cell line is an example of Claudin low subtype with a low expression of Ki67, E-cadherin, claudin-3, claudinin-4 and claudinin-7, while MCF-7 represents the luminal breast cancer cell line, with expression of ER, HER2 negative, PR expression or not and low Ki67 [9,10]. Despite the therapeutic and molecular advances of recent years, there is no validated target to be blocked in the triple negative subset and chemotherapy is the only available systemic therapy [11]. Currently, taxane and anthracycline-based combination chemotherapy remains the standard treatment approach for triple negative subtype, and this approach has not changed much in the last decade [[17],12,13]. Doxorubicin is an anthracycline antibiotic

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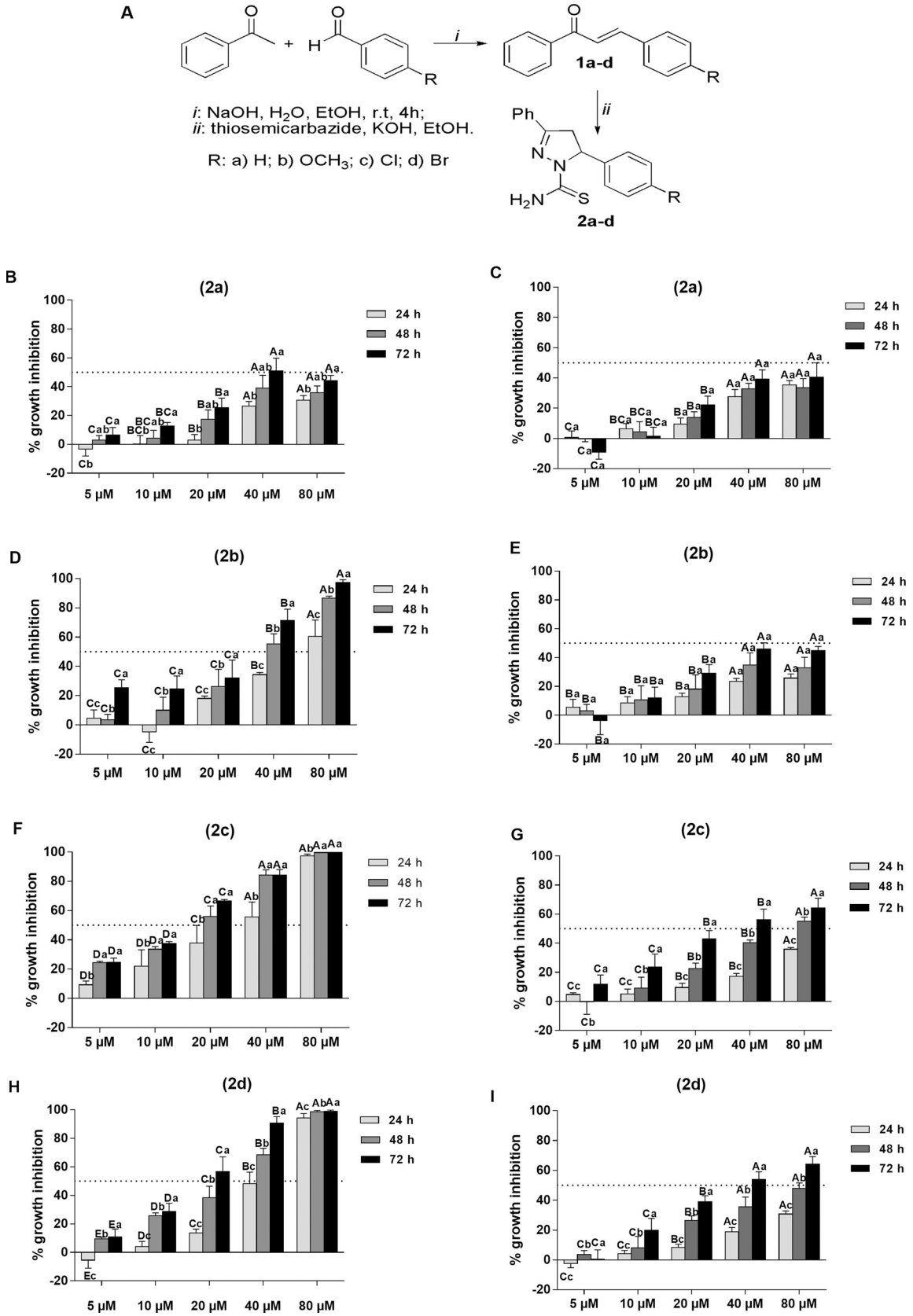

Fig. 1. A: Synthesis of 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-1H-pyrazoles **2a–d**; **1B:** Effect of the pyrazoles, (2a), (2b), (2c), (2d), on cell viability in human breast cancer MDA-MB-231(B,D,F,H) and MCF-7 (C,E,G,I) cells, using the MTT assay. Cells were treated with concentrations of 5, 10, 20, 40, and 80 μM for 24, 48 and 72 h. Values are presented as mean ± SEM of three independents experiments performed in triplicate. Uppercase letters represent concentration and lowercase letters represent time. Different letters indicate significant differences among means. Significance was considered at $p < .05$.