

Chemoselective synthesis, antiproliferative activities, and SAR study of 1H-pyrazol-5-yl-N,N-dimethylformamidines and pyrazolyl-2-azadienes

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Abstract: Chemoselective microwave-assisted amidination was successfully developed to synthesize 1*H*-pyrazol-5-yl-*N,N*-dimethylformamidines and pyrazolyl-2-azadienes. All of the starting materials and resulting products were tested against NCI-H226, NPC-TW01, and Jurkat cancer cells to evaluate their antiproliferative activities.

1*H*-Pyrazol-5-yl-*N,N*-dimethylformamidines 2b, 2c, and 2d were most potent with IC₅₀ values in low micromolar range. The formyl group at C-4 position and the grafted amidinyl group in the main core of pyrazolic molecule were necessary for the inhibitory activity.

Upregulating Noxa by ER stress, Celastrol exerts synergistic anti-cancer activity in combination with ABT-737 in human hepatocellular carcinoma cells

Abstract: A simple and rapid method was developed for the determination of celastrol in standard and human hepatocellular carcinoma cells. The chromatographic separation was accomplished on an YMC-Pack ODS column (2.0 mm×100 mm) at 30 °C. The detection wavelength was set at 280 nm. The reference standard was celastrol. The detection limit was 0.05 μg/ml. The precision was acceptable. The accuracy was 97.2%–102.8%. The recovery was 96.2%–103.8%. The method was applied to the determination of celastrol in human hepatocellular carcinoma cells. The results showed that celastrol could induce apoptosis in human hepatocellular carcinoma cells. The mechanism of celastrol-induced apoptosis may be related to the upregulation of Noxa expression. The results indicated that celastrol could exert synergistic anti-cancer activity in combination with ABT-737 in human hepatocellular carcinoma cells.

肝细胞癌是一种恶性程度较高、易发生耐药的肿瘤，严重威胁病人的生命。Bcl-2 抑制剂 ABT-737 现处于临床研究 II 期，由于其和抗凋亡蛋白 Mcl-1 的低亲和力，往往在 Mcl-1 表达的肿瘤（包括肝细胞癌）中疗效欠佳。本研究将 ABT-737 与天然来源的抗肿瘤活性化合物雷公藤红素合用，发现其具有协同抑制人肝癌细胞 Bel-7402 和 HepG2 生长的作用，合用指数 (CI) 均小于 0.7；采用 DAPI 染色、PI 单染、JC-1 染色结合流式细胞术发现 ABT-737 和雷公藤红素能协同诱导 Bel-7402 和 HepG2 细胞凋亡的发生，伴随着 Caspase 级联反应的激活和细胞色素 C 从线粒体中释放。机制研究显示雷公藤红素能诱导促凋亡蛋白 Noxa 的 mRNA 和蛋白表达的增加，且该上调作用的发生早于细胞凋亡；免疫共沉淀实验显示雷公藤红素能显著促进 Noxa 与 Mcl-1 的结合；而将 Noxa 敲除后，则显著减少合用后导致的肿瘤细胞增殖抑制以及凋亡发生，提示 Noxa 的上升能阻断 Mcl-1 的抗凋亡活性，是发挥协同抗肿瘤作用的重要原因之一。进一步研究发现，雷公藤红素能诱导 Bel-7402 和 HepG2 细胞内 Ub 上调，HSP90 客户蛋白 p-ERK, CDK4 降解，HSP70 蛋白累积，具有 HSP 抑制活性，与文献报道一致；进而我们发现雷公藤红素能够引发内质网应激反应，诱导 p-eIF2a 和 ATF4 上调；敲除 ATF4 基因以后，与未敲除的细胞相比，雷公藤红素上调 NOXA mRNA 表达的作用明显减弱，提示雷公藤红素是通过其 HSP90 靶向作用，导致内质网应激的发生，从而通过 ATF4 调节 Noxa 表达的。因此我们的研究为将雷公藤红素和 ABT-737 作为治疗肝细胞癌的有效合用组合提供了