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Original article

Directional modification of chrysin for exerting apoptosis and enhancing significantly anti-cancer effects of 10-hydroxy camptothecin

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ABSTRACT

Chrysin, one of natural flavonoid compounds, has recently been found to possess anti-inflammatory, antiallergic and anticancer properties. To increase its anticancer effects, 5 chrysin derivates were synthesized on the base of DNA intercalator structure. The inhibiting effects of chrysin and its derivatives on cancer cells Hela, BGC823, MCF-7, HepG2, and normal cells HEK-293, were evaluated by MTT assays. 5-(2′-amino) phenyl-7-cyclohexanemethylchrysin (Ch-1), a unique chrysin derivate, killed all the cancer cells but kept above 60% survival rate in normal cells HEK-293 at 62.5 μM. Treated with chrysin from 250 μM to 500 μM, those cells were still maintained above 60% survival rate. The result of circular dichroism spectra showed that Ch-1 could intercalate DNA while chrysin had no effects on DNA. Interestingly, Hela cells survival rates were 95% and 10%, after treated with 20 μM and 30 μM of Ch-1, respectively. Both intrinsic and extrinsic apoptotic pathway were identified in regulating the cell death caused by Ch-1 in Hela cells. p53, the upstream regulator of apoptotic pathway were extremely significantly up-regulated in Hela cells treated with 25 μM Ch-1. Moreover, the inhibiting effects and apoptotic related proteins responses to Ch-1 on Hela cells were abolished after pre-treated with Pifithrin-α (Pft-α), a p53 inhibitor. So, p53-depedent apoptosis is the crucial factor governing the inhibiting effects of Ch-1 in Hela cells. Amazingly, Ch-1 at non-toxic concentration (2.5–10 μM) enhanced significantly anti-cancer effect of 10-hydroxy camptothecin (HCPT) on Hela, BGC823, and MCF-7 cells.

1. Introduction

Chrysin (5,7-dihydroxyflavone, Fig. 1) is one kind of phenolic compounds called flavones, and widely exist in plant extracts, honey and propolis. Chrysin has been reported to exert various pharmacological activities including antioxidant, anti-inflammatory, anti-diabetic and anticancer [1,2]. However, the anticancer activity was low. It is reported that metallic complex of chrysin presents 10-fold higher anti-cancer activity than chrysin through helping chrysin insert into hydrophobic regions of DNA [3]. Analyzing the structure of La (III) complex of chrysin, it is found that the introduction of La(III) is like to introduce positively charged benzene ring to 5′-OH. So, introduction of aniline group to 5′-OH may replace the role of La(III). Considering the structure of DNA, negatively charged property of 7′-OH may interfere the interaction between chrysin derivate and DNA, as well as the derivates were inserted to hydrophobic regions of DNA, so some hydrophobic group were introduced to 7′-OH.

DNA intercalators are an important and effective type of anti-cancer agents, which bind to DNA to change DNA replication and inhibit the growth of tumor cells. Apoptosis, or the process of programmed cell death, is considered to be a vital component of chemical-induced cell death. There mainly exist two pathways, intrinsic and extrinsic, to activate apoptosis [4,5]. The intrinsic pathway, so-called “mitochondrial pathway”, is primarily regulated by Bcl-2 family which have classically been divided into three groups: anti-apoptotic proteins (e.g. Bcl-2, Bcl-w, Mcl-1),

Abbreviations: Ch-1, 5-(2′-amino) phenyl-7-cyclohexanemethylchrysin; CD, circular dichroism; DMEM, Dulbecco’s Modified Eagle Medium; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; GAPDH, glyceraldehyde-phosphate dehydrogenase; HCPT, 10-hydroxy camptothecin; MMP, mitochondrial membrane potential; MTT, methyl thiazolyl tetrazolium; p53, apoptosis p53 protein; Pft-α, Pifithrin-α; PBS, sodium phosphate buffer; PI, propidium iodide; PVDFmembrane, polyvinylidene fluoride membrane; Rh123, Rhodamine 123; TBST, Tris-buffer with sodium and Triton X-100 (20 mM Tris–HCl 120 mM NaCl and 0.1% (v/v) Tween 20).

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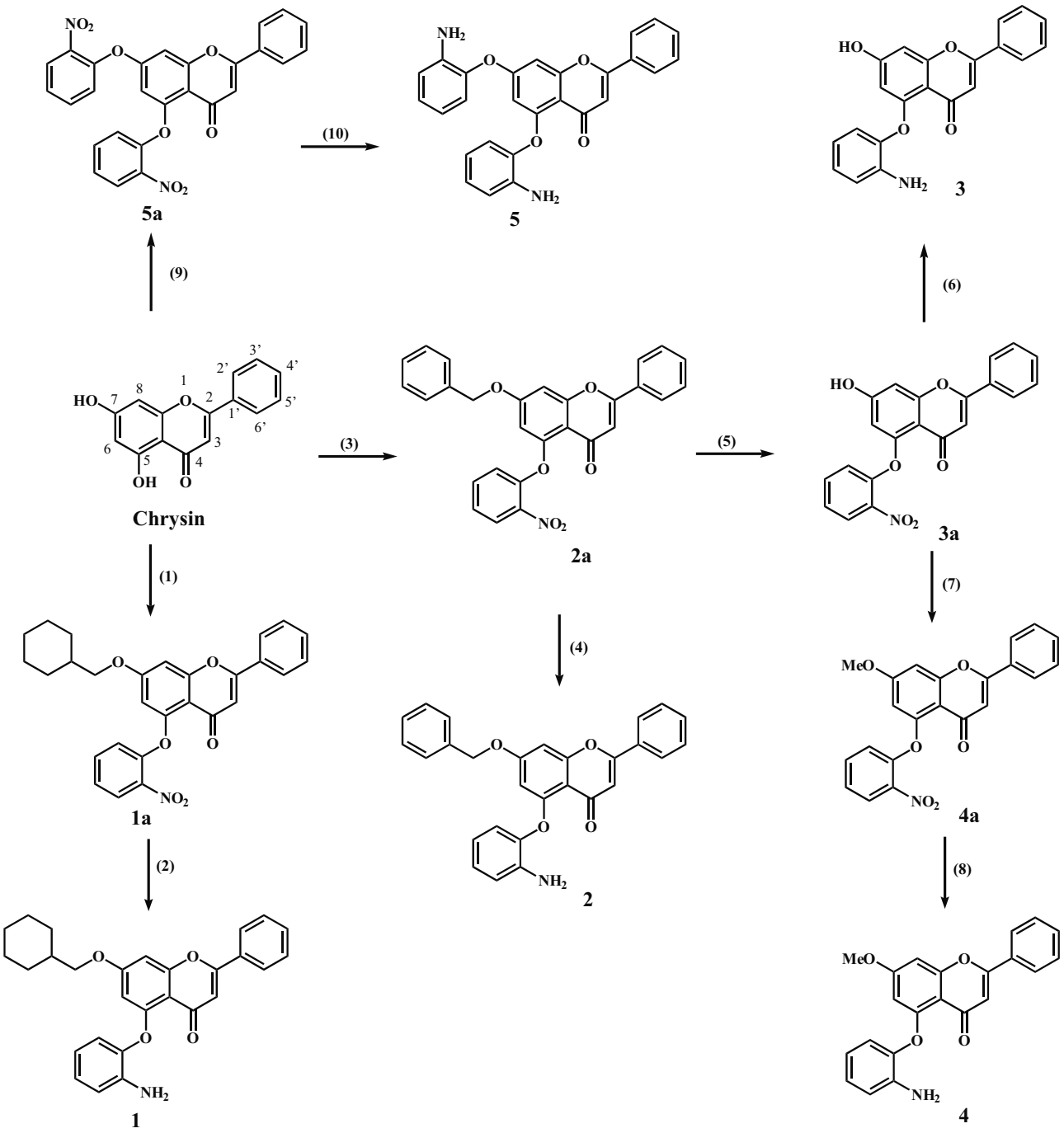

Fig. 1. Synthetic paths of chrysin derivatives. (1) Cyclohexylmethyl bromide, 1-fluoro-2-nitrobenzene, K_2CO_3, NMP, 72%; (2) $SnCl_2{\cdot}2H_2O$, CH_3COOH, c-HCl, 80 °C, 2 h, 97%; (3) Benzyl bromide, 1-fluoro- 2-nitrobenzene, K_2CO_3, NMP, 99%; (4) $SnCl_2{\cdot}2H_2O$, CH_3COOH, 80 °C, 2 h, 80%; (5) c-HCl, CH_3COOH, 80 °C, 12 h, 99%; (6) $SnCl_2{\cdot}2H_2O$, CH_3COOH, c-HCl, 80 °C, 2 h, 69%; (7) MeI, K_2CO_3, NMP, 75 °C, 1 h, 92.5%; (8) $SnCl_2{\cdot}2H_2O$, CH_3COOH, c-HCl, 80 °C, 3 h, 72%; (9) 1-fluoro-2-nitrobenzen, K_2CO_3, NMP, 80 °C, 95.8%; (10) $SnCl_2{\cdot}2H_2O$, CH_3COOH, c-HCl, 80 °C, 4 h, 97.7%.

pro-apoptotic proteins (e.g. Bak, Bax, Bok), as well as BH3 only proteins (e.g. Bid, Puma, Bad) [6–9]. After apoptotic stimuli, e.g., by chemotherapy drugs, the imbalance of pro/anti-apoptotic proteins will increase the permeability of mitochondrial membrane, lose the transmembrane potential and release cytochrome c (Cyto c) [10]. Cyto c forms a complex with apoptotic protease-activating factor (Apaf-1) to activate caspase-9 [11,12]. Cleaved-caspase-9 will induce apoptosis by activating caspase-3/6/7. On the other hand, the extrinsic pathway is regulated by tumor necrosis factor (TNF) receptor including TNF receptor, Fas and TNF-related apoptosis-inducing ligand (TRAIL) such as DR5 and DR4 [13,14]. After binding of death receptor and corresponding ligand, pro-caspase-8/10 is cleaved to directly activate caspase-3/6/7 or induce the intrinsic signaling pathway by cleaving Bid [15], which will trigger mitochondrial membrane permeabilization via the activation of Bax and Bak [16].

p53 is the first tumor suppressor gene linked to apoptotic pathway. In terms of extrinsic apoptotic pathway, Fas and DR5 have been indentified as targets for p53 transcription [17]. Moreover, p53 can transactivate a subset of key genes involved in mitochondrial and post-mitochondrial apoptosis signaling pathways involving Bax and Bak, Puma and Noxa, and Apaf-1 to