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# New bichalcone analogs as NF-κB inhibitors and as cytotoxic agents inducing Fas/CD95-dependent apoptosis

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ABSTRACT

A series of novel bichalcone analogs were synthesized and evaluated in lipopolysaccharide (LPS)-activated microglial cells as inhibitors of nitric oxide (NO) and for in vitro anticancer activity using a limited panel of four human cancer cell lines. All analogs inhibited NO production. Compounds **4** and **11** exhibited optimal activity with $IC_{50}$ values of 0.3 and 0.5 μM, respectively, and were at least 38-fold better than the positive control. A mechanism of action study showed that both compounds significantly blocked the nuclear translocation of NF-κB p65 and up-regulation of iNOS at 1.0 μM. Compound **4** and three other analogs (**3**, **20**, and **23**) exerted significant in vitro anticancer activity $GI_{50}$ values ranging from 0.70 to 13.10 μM. A mode of action study using HT-29 colon cancer cells showed that **23** acts by inducing apoptosis signaling.



## 1. Introduction

Dietary flavonoids, commonly present in edible plants, are known to have beneficial effects, such as antioxidative effects, tumor cell growth inhibitory activity, and apoptosis induction in cancer cell lines. Therefore, dietary flavonoids have attracted attention as chemopreventive agents.[1] Chalcones are the immediate precursors in the biosynthesis of flavonoids, and their structure differs considerably from the other members of the flavonoid family. Chalcones are reported with diverse biological activities including anti-inflammatory, anti-malarial, anti-protozoal, anti-bacterial, nitric oxide inhibition, tyrosinase inhibition, cytotoxic, anticancer, and anti-leishmanial activities.[2–6] The bichalcones are well represented in the Anacardiacea family. The *Rhus* genus is also a rich source for biflavonoids and bichalcones. In general, naturally occurring bichalcones carry either C–O–C or C–C linkage between the two chalcone units. Although natural bichalcones from *Rhus pyroides* demonstrated varying degrees of cytotoxic activity against different cancer cell lines, they showed more selectivity toward colon cancer cell lines, especially the HT29 and HCT-116 cell lines.[7]

The Mannich base reaction is an important carbon–carbon bond-forming reaction in organic synthesis, and it has been widely utilized in the synthesis of nitrogen-containing drugs, natural products and biologically active compounds.[8,9] Considering the pharmacological importance of bichalcones, we synthesized a series of bichalcone analogs through the piperazine Mannich base linkage with different substitutions in the B-ring of the chalcone moiety. Target compounds were examined as inhibitors of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated microglial cells, which are important free radical-producing cells in the central nervous system. Rapid production of reactive oxygen species (ROS) by NADPH oxidase (NOX) and nitric oxide (NO) by NO synthase (NOS) can be generated experimentally.[10] Thus, we investigated the expression of iNOS protein and NF-κB p65 (C) and p65 (N) in presence of bichalcone analogs. In addition, we further evaluated the in vitro anticancer activity of the newly synthesized compounds against four human cancer cell lines, and explored the mechanism of action of a selected compound (**23**) in the HT-29 human colon adenocarcinoma cell line.

## 2. Chemistry

4-Hydroxyacetophenone (**1**) and 4-hydroxy-3-methoxyacetophenone (**2**) were reacted with 4-piperazinoacetophenone and

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