New Synthetic Flavonoid with *in vitro* Antitumor Activity

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Abstract— Flavonoids have a wide spectrum of biological activities like antioxidant, anti-inflammatory, antitumor, etc. In this work a novel synthetic flavonoid ''ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate'' was synthesized, characterized, and then investigated *in vitro* for potential antitumor activity in compare to that of the well established antitumor natural flavonoid ''quercetin''. The new compound displayed potent antiproliferative action against human prostate cancer (PC3) and human hepatoma (HEPG2) cells. It can be concluded that the new synthetic flavonoid have *in vitro* optimistic antitumor activity.

Index Terms— Breast cancer, Colorectal carcinoma, 4H-benzo(h)chromene-3-carboxylate, Hepatoma, Prostate cancer, Tumor.

1 Introduction

Tumor is an uncontrollable growth of abnormal cell [1]. Oxidative stress, imbalance between oxidants and antioxidants [2], is behind many pathologic conditions such as liver [3], [4] and kidney [5], [6] dysfunction and it is a major contributor in tumorgenesis process as well [7], [8]. Free radicals are able to motivate cell mutations [1] and they can cause damage to many vital celluar components [9], [10], [11], [12]. Therefore, natural or synthetic molecules with antioxidant properties are optimistic potential candidates in tumor prevention and cure.

Tumors are a major leading causative factor of death worldwide [13], [14], [15], [16]. Globally, they recorded about 7.6 million deaths in 2005 that are expected to exceed 11 millions in 2030. Nearly, three quarters of tumor-linked deaths are associated with Developmental Countries [17]. Although scientists great efforts, tumor drugs that are successful and satisfied for both clinicians and patients are not developed yet. This makes discovering of novel antineoplatic molecules on the top of researchers interests [18].

Natural products/natural product-derived antitumor molecules represent more than two-thirds of the studied antitumor agents. From diverse antitumor and prophylactic agents, flavonoids are the widely investigated group. Flavonoids have the ability to obstruct particular steps of tumorgenesis, limit tumor cells proliferation, and motivate programmed cell death in many different neoplasms. Flavonoids exhibit appreciated antioxidation properties; they prevent generation of free radicals and influence various detoxifying enzymes activities as well. Flavonoids antitumor power may be attributed to their antioxidation properties [19].

Quercetin is a very common natural flavonoid present in considerable concentrations in virtually all edible vegetables and fruits. Numerous scientific investigations evidenced that quercetin has pronounced prophylactic and curative abilities towards numerous chronic diseases including various tumors [20].

This study was proposed to synthesize a novel synthetic flavonoid and to investigate its possible antitumor activity *in vitro*, compared to the well known antitumor natural flavonoid quercetin, against four human cancer cell lines.

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2 Materials and Methods

2.1 Chemicals and Cell Lines

MTT and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylcyanoacetate, benzaldehyde, α-naphthol, Na_2CO_3 were purchased from TEDIA Company, USA. The rest of chemicals were of analytical grade.

Human mammary gland (MCF-7), colorectal carcinoma (HCT-116), hepatoma (HEPG2), and prostate cancer (PC3) cell lines were got from VACSERA Company, Cairo, Egypt.

2.2 Synthesis of the Novel Synthetic Flavonoid

A novel synthetic flavonoid (ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate) (Fig. 1) was synthesized by an efficient, solvent-free one-pot three-component cyclo-condensation. Ethylcyanoacetate, benzaldehyde and α-naphthol then Na_2CO_3 were placed in fusion oil path for 15-25 min at the 100-120 °C, precipitated with ice-water bath and the obtained brown precipitate was filtered. Recrytallization was performed using EtOH. Yield: 67% and m.p. is 165-170 °C.

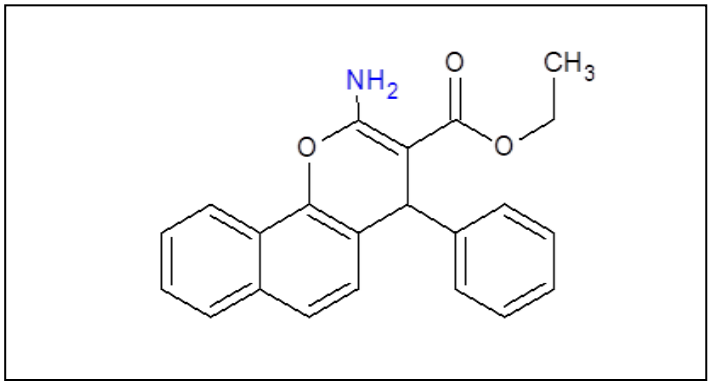

Fig. 1. Structure of the new synthetic flavonoid ''ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate''.

2.3 Characterization of the Novel Synthetic Flavonoid

IR spectroscopy analysis was done using FT-IR spectra (KBr discs, 4000–400 cm-1) by Jasco-4100 spectrophotometer, at the (IR) unit at Faculty of Science, Damietta University, Egypt. Total organic carbon (TOC) analysis was done at Faculty of Science, Kafr El-Sheikh University, Egypt.

Mass spectrum analysis was done at Faculty of Science, El-Azhar University, Egypt.

2.4 Cytotoxicity Test

Inhibitory effects on cell growth (proliferation) *in vitro* for our new compound were assayed using MTT method and quercetin was used as reference. Yellow tetrazolium bromide (MTT) is changed to purple formazan derivative in alive cells in the presence of mitochondrial succinate dehydrogenase. Cancer cell lines were grown in RPMI-1640 medium in presence of fetal bovine serum (10%), penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37 °C in 5% carbon dioxide incubator. Cancer cell lines were seeded ($1x10^4$ cells/well) at 37 °C for 48 h under 5% CO_2. After this, the cells were treated with variable doses of investigated compounds and incubated for 24 h. Then, MTT (20 µl of 5 mg/ml solution) was added and incubated for 4 h. DMSO (100 µl) was added to each well to solubilize purple formazan. Optical density (O.D.) was read at 570 nm. IC_{50} values were determined.

The study protocol was approved by the Chemistry Department, Faculty of Science, Damietta University.

2.5 Statistical Analysis

Analyses were performed using the statistical package for social science "SPSS" 22.0 for Microsoft Windows, SPSS Inc., Chicago, USA. Values were introduced as mean±SEM. P value < 0.05 was considered statistically significant.

3 Results

3.1 Spectral analyses of the Novel Synthetic Flavonoid

IR (KBr. cm^{-1}): 3487 (NH_2), 3057 (Ar-H), 1658 (C=O), 1588 (C=N), 1563 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 6.82 (d, 2H, NH_2), 7.37-8.27 (m, 1H, Ar-H), 4.74 (s, 1H, CH), 4.08 (q, 2H, CH_2), 1.16 (t, 3H, CH_3). m/z: 345.14 (100.0%), 346.14 (23.8%), 347.14 (2.7%). Anal. Calcd. C, 76.50; H, 5.54; N, 4.06; O, 13.90. Found: C, 75.40; H, 6.04; N, 4.56; O, 13.20. Chemical formula is $C_{22}H_{19}NO_3$, and Mwt. is 345.40.

3.2 Cytotoxicity

Fig. 2 to Fig. 5 displayed gradual decrease in the number of alive human tumor cells as the compound dose increases from 0.0 to 50 µg/ml.

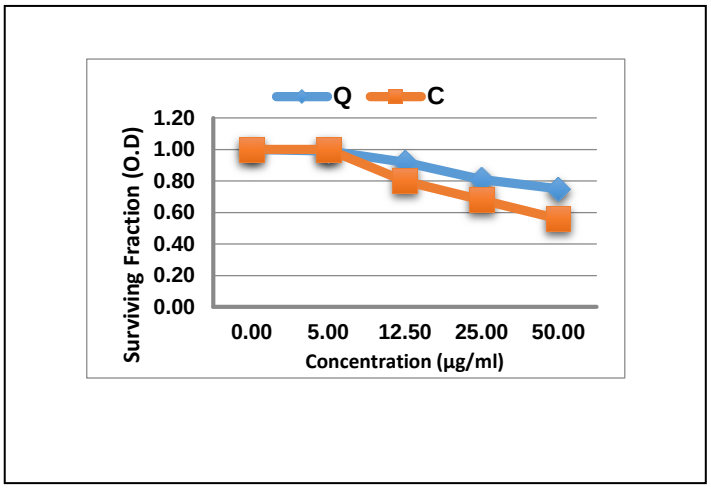

Fig. 2. Concentration-dependent cytotoxicity against human mammary gland (MCF-7) cell line. *C: the new compound. Q: quercetin.*

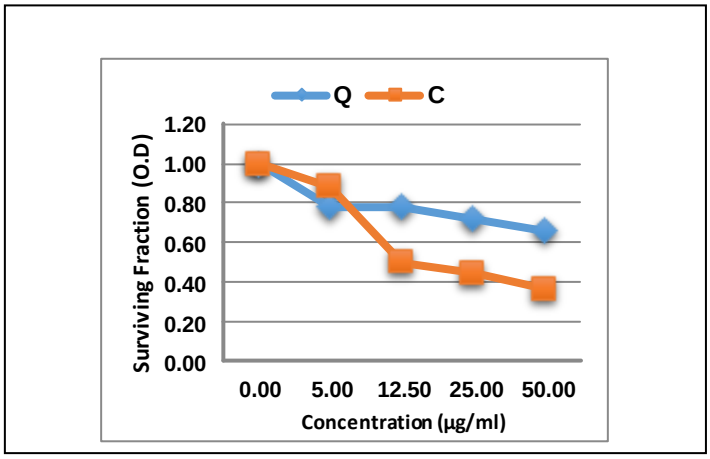

Fig. 3. Concentration-dependent cytotoxicity against human human colorectal cancer (HCT-116) cell line. *C: the new compound. Q: quercetin.*

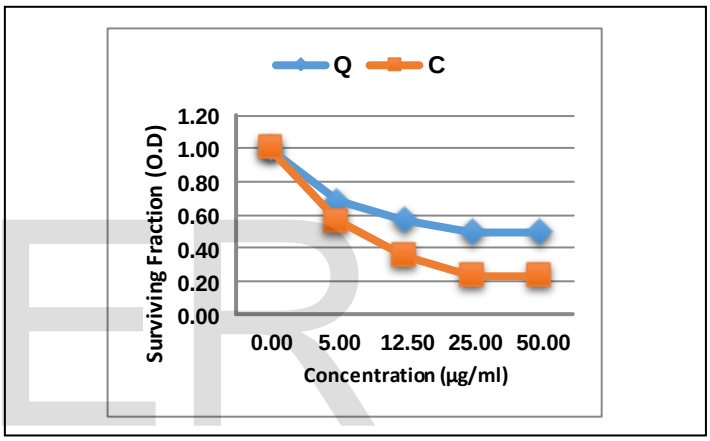

Fig. 4. Concentration-dependent cytotoxicity against human hepatoma (HEPG2) cell line. *C: the new compound. Q: quercetin.*

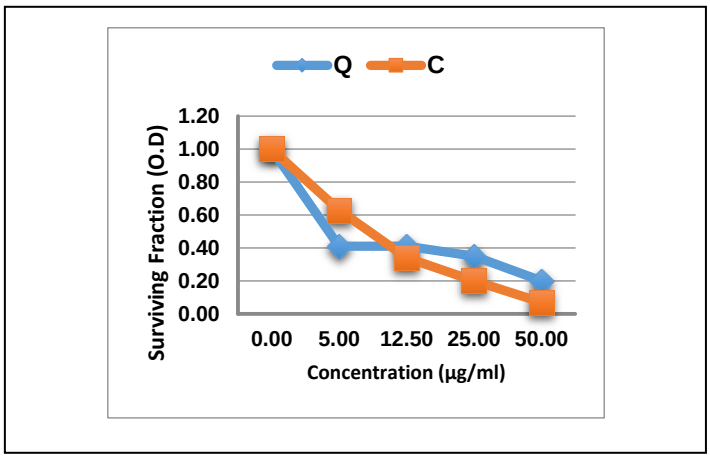

Fig. 5. Concentration-dependent cytotoxicity against human prostate cancer (PC3) cell line. *C: the new compound. Q: quercetin.*

Table 1 showed that the new compound showed no antiproliferation activity against HCT-116; it was considered non-toxic with IC_{50} value more than 100 µg/ml and reference compound quercetin was considered weak toxic with IC_{50} value more than 50 µg/ml.

Both new compound and quercetin showed weak antiproliferation activities (IC_{50}: >50 µg/ml) against MCF-7. Considering HEPG2 cells, the new compound showed very strong toxicity with IC_{50} equals 7.0 µg/ml while quercetin showed moderate toxicity with IC_{50} equals 22.5 µg/ml. Regarding PC3, both new compound and quercetin showed very strong antiproliferation activities (IC_{50}: 4.0 µg/ml and 8 µg/ml, respectively).

TABLE 1
IC_{50} against human cell lines

Cell line	C	Q
MCF-7	>50±1.52	>50±1.84
HCT-116	>100±2.67***	>50±1.79
PC3	8.0±0.45**	4.0±0.62
HEPG2	7.0±0.32***	22.5±1.12

*C is the new compound, Q is quercetin, MCF-7 is human mammary gland, HCT-116 is human colorectal carcinoma, HEPG2 is human hepatoma, and PC3 is human prostate cancer cell lines. IC_{50} (µg/ml): 1 – 10 is very strong, 11 – 20 is strong, 21 – 50 is moderate, >50 – 100 is weak, and >100 is non-cytotoxic. Data presented as Mean±SEM. ** is P < 0.01, and *** is P < 0.001 versus Q.*

There was no significant (P > 0.05) difference between the new compound and quercetin in IC_{50} values against breast cancer. While, there were significant increases in IC_{50} values of the new compound against both prostate cancer and colorectal carcinoma (P < 0.01, and P < 0.001, respectively). On contrary, there was a significant decrease in IC_{50} value of the new compound against hepatoma (P < 0.001) (Table 1).

4 Discussion

In the current work, a novel synthetic flavonoid ''ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate'' was successfully synthesized by the efficient, solvent-free one-pot three-component cyclocondensation method. This procedure is simple, safe, easy, rapid, and give high yield [21]. Synthesized compound was chemically characterized using IR, NMR and mass spectroscopy. Spectral data presented in results section elucidate the new flavonoid suggested structure in Fig. 1.

Antitumor activity of natural flavonoid quercetin is widely studied as we referred above and it was stated that chronic consumption and daily administration of quercetin might be useful for avoidance of some types of neoplasms. For example, quercetin was found to prevent tumor progression in cancer cell lines of mammary gland, stomach, lung, colon, ovary, head, and others [19].

In the present study, the *in vitro* cytotoxic activity of the new synthetic flavonoid was evaluated in compare to that of quercetin, as reference. Both displayed gradual decrease in the number of survive human tumor cells [mammary gland (MCF-7), colorectal carcinoma (HCT-116), hepatoma (HEPG2) and prostate cancer (PC3)] as their doses increased from 0.0 to 50 µg/ml. This suggests concentration-dependent cytotoxic activities. This finding is in accordance with findings of [1], [8], [17], and [18]. They concluded that drop in viable cancer cells number by treatment with a specific molecule suggests antitumor activity of that molecule.

Among the studied tumor cell lines, the new compound was very strong cytotoxic against both hepatoma (IC_{50} = 7.0 µg/ml) and prostate cancer (IC_{50} = 8.0 µg/ml) while it was weak cytotoxic against breast cancer (IC_{50} > 50 µg/ml) but it was considered non-toxic against colorectal carcinoma (IC_{50} > 100 µg/ml). It is worthy to mention that the new compound was more stronger antiproliferative than quercetin against hepatoma, was nearly of equal power with quercetin against prostate and breast cancers, and was less powerful than quercetin against colorectal carcinoma. These results suggest that the new compound have a potent antitumor activity against both liver (HEPG2) and prostate (PC3) cancers *in vitro*.

Previously, many 2-amino-4H-chromenes were investigated for treatment of a great variety of tumors and they displayed very beneficial proapoptotic activities. 2-amino-4H-chromene was indicated for chemotherapeutic drug development owing to its great suppression of tumor-associated Bcl-2 proteins [21]. Moreover, another modified 4H-chromene structure was seen to be able to induce apoptosis in several cancer cell lines [22].

5 Conclusion

Study results introduce ethyl 2-amino-4-phenyl-4H-benzo(h)chromene-3-carboxylate as a new synthetic flavonoid with *in vitro* potent antitumor activity against human hepatoma (HEPG2) and prostate (PC3) cancer cell lines. Further *in vivo* investigations are required to support our *in vitro* findings.

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