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Synthesis and Evaluation of Antibacterial and Antitumor Activities of Apigenin Derivatives

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Using nicotinic acid, isonicotinic acid reacted with apigenin *via* DCC condensation to produce apigenin derivatives. Their chemical structure was confirmed by MS and ^{1}H NMR spectroscopy. The antimicrobial activities of the new compounds and raw materials were explored with *Staphylococcus aureus* and *Acinetobacter*. while the antitumor activities of the new compounds and raw materials were explored with SH-SY5Y, MCF-7, HepG2 under the same conditions. The results show that apigenin derivatives have stronger antibacterial properties on *Acinetobacter* than *Staphylococcus*. The new compounds have the strongest antitumor activities on HepG2, followed by MCF-7 and relative weak activities on SH-SY5Y *in vitro*.

Keywords: Apigenin derivatives, Antibacterial, Antitumor.

INTRODUCTION

Apigenin (5,7,4'-trihydroxyflavone) belongs to flavonoid, which is widely present in a variety of vegetables, herbs and celery. It has different apigenin content in different regions and different types of vegetables. According to the study, Hubei large leaf vegetables vegetarian water celery has the highest apigenin[1]. *In vitro* and animal studies found that apigenin has a variety of pharmacological activities: specific performance in antitumor, anti-inflammatory[2], anti-free radical[3], protect nerve damage and cerebral reperfusion injury, lowing blood pressure anxiolytic, antibacterial, antiviral and anti-oxidation, *etc*[4]. There are three hydroxyl groups and double bonds in apigenin. Its structure determines its unique physiological effects and biological characteristics[3]. But there were few reports about the active phenolic hydroxyl. Nicotinic is commonly used to adjust dyslipidemia drugs clinically[5], the study of isonicotinic is also very important. We designed and synthesized nicotinic acid and isonicotinic acid derivatives of apigenin.

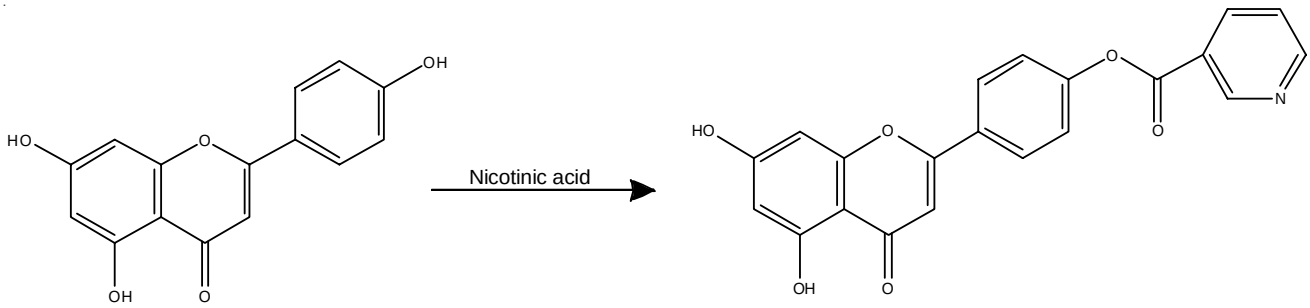
EXPERIMENTAL

Synthesis: The compounds are synthesized as according to the reported literature[6]. The synthesis of nicotinic acidified apigenin was carried out in dry $CHCl_2$ in the presence of dehydrating agents, 0.2 mmol nicotinic was mixed with 0.1 mmol apigenin and refluxed for 24 h using dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP) as catalysts. Then aqueous hydrochloric acid solution was used to remove the unreacted DCC and DMAP, afterwards separated the compounds using silica gel (100 mesh) column with methanol:carbon tetrachloride:acetic acid (1:10:1) and dried them respectively (**Scheme-I**). The synthesis of isonicotinic acidified apigenin was used the same method as that of nicotinic acidified apigenin (**Scheme-II**).

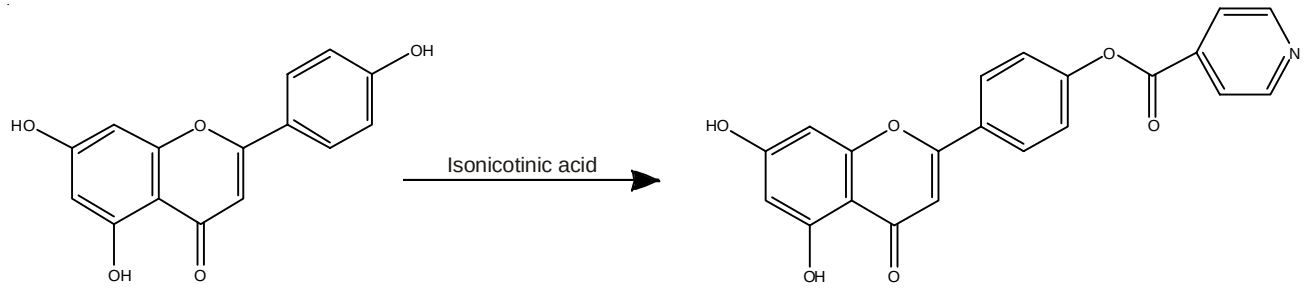
Nicotinic acidified apigenin: ^{1}H NMR (500 MHz, $CDCl_3$): δ5.35 (s, 2H, 2 × OH), δ 6.25-7.5 (m, 7H, ArH), δ 7.46 (m,1H), δ 8.67 (d, J = 10 Hz, 1H), δ 8.65 (d, J = 10 Hz, 1H), δ 8.88 (s, 1H). EIMS: M^+ *m/z* 375.

Isonicotinic acidified apigenin: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (s, 2H, 2 × OH), δ 6.25-7.5 (m, 7H, ArH), δ 8.92 (d, J = 15 Hz, 2H), δ 8.89 (d, J = 15 Hz ,2H). EIMS: M^+ *m/z* 375.

Antibacterial activity: The antibacterial activity was tested by Poke-Pole method[7]. *Staphylococcus aureus* and *Acinetobacter* were activated for 2 h at optimum temperature and then accessed to freshly prepared medium and shaking cultured for 24 h. The resulting broth was diluted to 10^8 cfu/mL. In the experiment, the sterile filtration of DMSO was in a solvent blank and positive control while the sterile saline was taken as a negative control. In each dish access 10^8 cfu/mL experimental strains for 100 mL and poke holes for 6 mm. Then add 10 µL apigenin derivatives solution, apigenin solution, nicotinic acid solution, sionicotinic acid solution, DMSO and sterile saline solution into the well, respectively. Each solution was three parallel experiments alone. They were



Scheme-I: Synthesis of nicotinic acidified apigenin. Reagents and conditions: nicotinic acid 0.2 mmol, apigenin 0.1 mmol, DCC 0.13 mmol, DMAP 0.05 mmol, 28 °C, reflux, for 24 h



Scheme-II: Synthesis of isonicotinic acidified apigenin. Reagents and conditions: isonicotinic acid 0.2 mmol, apigenin 0.1 mmol, DCC 0.13 mmol, DMAP 0.05 mmol, 28 °C, reflux, for 24 h

developed at an optimal incubation temperature for a growth cycle and then the diameter of each zone of inhibition was measured.

Antitumor activity: The antitumor activity was tested by MTT assay. MCF-7, SH-SY5Y, HepG2 cells were seeded into 96-well plates, respectively with about 1000-10000 cells in each well. The volume of each well was 100 uL and the screening concentration was 20 mM, located three complexed wells. The cells were cultured for 2 days and then add 15 μL MTT solution to the well, respectively. Incubation was continued for 4 h and then terminated. The culture supernatant was discarded smoking hole. 150 mL DMSO was added to each well and shocked for 10 min to dissolve the crystals sufficiently. The absorbance in each well was measured by enzyme-linked immunosorbent assay in the 570 nm. In the experiment, DMSO, TAX and noscapine were, respectively taken as positive control.

RESULTS AND DISCUSSION

Antibacterial activities: Table-1 showed that the new compounds have more antimicrobial activities on *Acinentober* than *S. aureus*. The ability of antimicrobial activity of apigenin derivatives on *Staphylococcus aureus* decreased while on *Acinetober* increased.

TABLE-1
INHIBITION ZONE OF COMPOUNDS ON BACTERIUM

	S. aureus	*Acinentober*
Apigenin	1.5	1.8
Nicotinic acid	1.4	1.6
Isonicotinic acid	1.45	4.2
Celery nicotinic acid derivative	1.32	6.03
Celery isonicotinic acid derivative	1.35	14.3
DMSO	1.1	1.1
Sterile saline	1.1	1.1

Fig. 1 indicates that both celery nicotinic acid and celery isonicotinic acid derivatives have much stronger antibacterial activities on *Acinentober* than celery. The celery isonicotinic acid derivative was about 13 times as good as celery, while celery nicotinic acid derivative was about 5 times as good as celery. All the compounds have nearly the same antibacterial activities on *Staphylococcus aureus*.

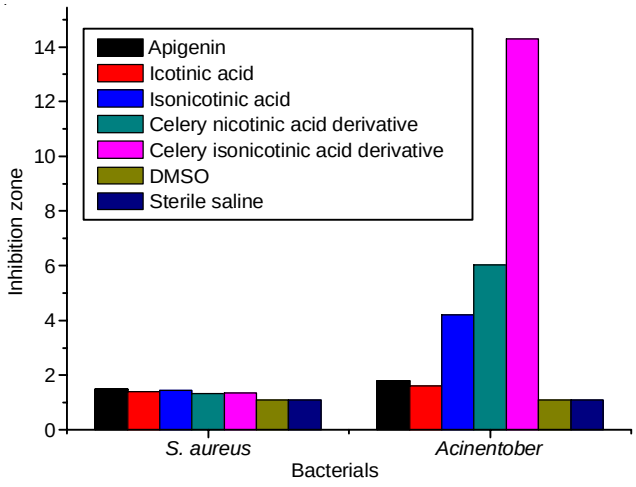


Fig. 1. Comparison of the relative antibacterial activities of the compounds

Antitumor activities: Table-2 showed that apigenin derivatives have the strongest effort on HepG2, followed by MCF-7, while they have little effect of antitumor onSH-SY5Y.

Fig. 2 gives the information that celery isonicotinic acid derivative has the strongest effort on MCF-7 than other compounds, it is also stronger than TAX; celery nicotinic acid derivative has the strongest effort on HepG2, it is about two times as good as noscapine. Both celery nicotinic acid derivative and celery isonicotinic acid derivative have much stronger effort on SH-SY5Y than celery.

TABLE-2
INHIBITION ROTE OF COMPOUNDS ON CELLS LINES

	MCF-7	SH-SY5Y	HepG2
Apigenin	0.372	0.09993	0.3126
Nicotinic acid	0.11382	0.14784	0.06903
Isonicotinic acid	0.25738	0.1407	0.0846
Celery nicotinic acid derivative	0.38213	0.32053	0.65041
Celery isonicotinic acid derivative	0.3865	0.3183	0.622
DMSO	-	-	0.449
TAX	0.352	-	-
Noscapine	-	0.249	-

Note: Data in table smaller, the ability of antitumor stronger

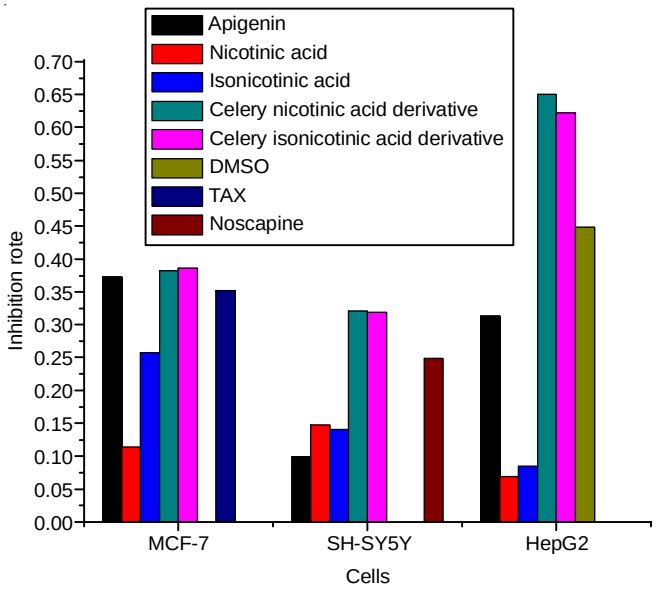


Fig. 2. Comparison of relative inhibition rote of compounds on the cell lines

Conlusion

Apigenin nicotinic acid derivative and apigenin isonicotinic acid derivative have specifically antibacterial such as *Acinentober* and antitumor activities such as HepG2. This study will be helpful and meaningful in the clinical application.

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