Synthesis of novel derivatives of curcumin as selective 11β-hydroxysteroid dehydrogenase 2 inhibitors

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Abstract

Colorectal cancer(CRC) becomes more and more common in our daily life, which has seriously affected on people's health. It has been demonstrated that inhibition of 11β-hydroxysteroid dehydrogenase type II (11β-HSD2) could significantly reduce cyclooxygenase 2 (COX-2) -mediated prostaglandin E2 (PGE2) production,which promoted CRC therapy. Curcumin has also been used as therapeutic agent of CRC, but is limited its applications for its low systemic bioavailability and poor absorption. In this study, three novel derivatives of curcumin (o-Cl**,** p-Br **and** p-CF3 **) were synthesized , examined on the inhibitory activity against 11β- HSD2 compared to curcumin. The IC50 of curcumin derivatives inhibition of on 11β- HSD2 in humam kidney microsome were 0.08±0.004 ,0.34 ±0.017 and 1.64±0.082, respectively, no significant difference among them, while significantly lower than curcumin, which was 34.5 ± 1.7.**

Keywords

11β-HSD2; Curcumin; Colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is one of the common gastrointestinal tract malignancies with high mortality rate. According to the cancer statistic in 2011, CRC is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cases and 0.6 million deaths estimated to have occurred worldwide[1]. It has been demonstrated that the progression of CRC was promoted by cyclooxygenase 2 (COX-2)-derived prostaglandin E_2 (PGE_2) and the inhibition of the expression of 11β-hydroxysteroid dehydrogenase type II (11β-HSD2) had a benefit impact on CRC therapy as well as prevented tumor formation, growth and metastasis[2-6].

11β-HSD2 is a NAD^+-dependent dehydrogenase with the function of converting cortisol to cortisone in kidney, colon, placenta and testis. Inhibition of 11β-HSD2 could significantly increase the level of glucocorticoid activity in colonic tumor, activate the receptor of glucocorticoid and thereby reduce COX-2-mediated PGE_2 production. So far, several selective 11β-HSD2 inhibitors derived from glycyrrhetinic acid have been synthesized and showed improving activity of inhibition.

Curcumin, a polyphenol and derived from *Curcuma longa* Linn, has been used as therapeutic agent for various types of cancer including CRC[7]. A number of pre-clinical and clinical trials revealed that curcumin blocks the transformation, proliferation and invasion of colon cancer by down-regulating COX-2[8]. Furthermore, it is well known that curcumin is safer and more tolerated than glycyrrhetinic acid in oral administration[9].

Although curcumin possesses potent property of anti-colorectal cancer and easy manipulation, low systemic bioavailability and poor absorption as well as rapid elimination limit its clinical applications[10]. To solve these problems, one of candidate methods is to enhance anti-carcinogenic activity of compounds. In the present study, we synthesized 3 novel curcuminoid drugs carrying

halogen moieties and investigated their inhibitory activity against 11β-HSD2 with comparison of curcumin.

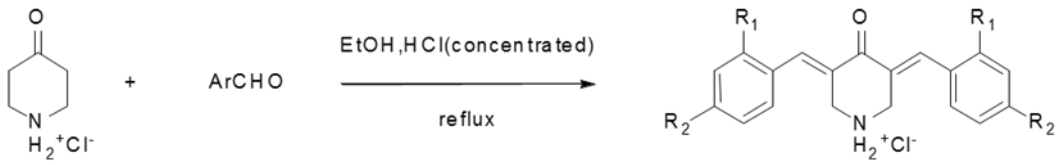
2. Experimental Detail

2.1 Materials

[1,2,6,7-N-^{3}H] Corticosterone (^{3}H-CORT, specific activity, 88 Ci/mM) was purchased from Dupont-New England Nuclear (Boston, MA). [^{3}H]11-dehydrocorticosterone (^{3}H-11DHC) was prepared from labeled ^{3}H-CORT as described previously. Unlabelled CORT and 11DHC were purchased from Steraloids (Newport, RI). Other reagents and solvents, including 4-Piperidone monohydrate hydrochloride, 2-Chlorobenzaldehyde, 2-Bromobenzaldehyde, 2- (Trifluoromethyl) benzaldehyde, 4-Fluorobenzaldehyde, 4-Chlorobenzaldehyde, 4-Bromobenzaldehyde, 4- (Trifluoro methyl) benzaldehyde and Hydrochloric acid as well as Ethyl Alcohol, were purchased from Sigma-Aldrich company. Human kidney microsomes (Cat. X03801, 10mg) was obtained from In Vitro Technologies company (Leipzig, Germany).

2.2 General Method for Synthesis of Curcumin Derivatives

The chromatography was performed on 230–400 mesh silica gels with technical grade solvents. ^{1}H- and ^{13}C-NMR spectra were recorded on a NMR 400 MHz spectrometer operated with the (Bruker) software. NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO). Chemical shifts are reported as δ values in parts per million (ppm) relative to the residual solvent peak and coupling constants are reported as J values in Hertz (Hz). IR spectra were recorded with AVATAR 360 FT-IR(Nicolet). High resolution mass spectra were obtained on a Reflex IV Bruker time-of-flight High-Resolution Mass Spectrometer (HRMS). Melting points were determined using a Differential Scanning Calorimeter (Ta,USA). The amount of curcumin analogues were determined using an Agilent 1200 HPLC system (Agilent, CA, USA) equipped with an Alltech Alltima C18 column (150 × 4.6 mm i.d., 5μ, Grace, IL, USA).



o-Cl:R1=H,R2=cl p-Br:R1=Br,R2=H p-CF3:R1=CF3,R2=H

Fig. 1 The synthesis scheme of curcumin derivates

1) **o-Cl**

The synthesis of Curcumin derivatives was summary in Fig.1.

The parent compound 4-Piperidone monohydrate hydrochloride, (1.0g,7.5mM) and 2-Chlorobenzaldehyde (2.11 g, 15 mM) added into a round bottom flask were dissolved with 10 ml ethyl-alcohol. Concentrated hydrochloric acid (5 ml, 11.9 M) was added into the resulting solution slowly accompanied with stirring at 0 ℃. Then the mixture was heated and refluxed for 6 h on a rotary evaporator until the final volume of solution was approximate 10 ml. The crude product was allowed to stand at room temperature for 3 h, filtered, washed 3 times with ethyl alcohol, and dried in a desiccator to give 1.8 g of the corresponding curcuminoids as yellow solids. Yield 68 %; ^{1}H NMR (300 MHz, DMSO) δ 10.00 (s, 2H), 7.97 (s, 2H), 7.78 – 7.57 (m, 2H), 7.57 – 7.30 (m, 6H), 4.35 (s, 4H); ^{13}C NMR (75 MHz, DMSO) δ 181.97, 135.68, 133.91, 131.53, 131.45, 130.63, 129.82, 129.54, 127.40, 43.63; EIMS m/z: 343(M-HCl); HREIMS Calcd. for $C_{19}H_{15}O_1N_1Cl_2$: 343.0525; Found: 343.0526.

2) **p-Br**

4-Bromobenzaldehyde (2.78 g, 15 mM) was used to synthesize instead of 2-Chlorobenzaldehyde. The method and the procession were the same as 3.2.1 description. Yield 61%; ^{1}H NMR (300 MHz, DMSO) δ 9.86 (s, 2H), 7.82 (s, 2H), 7.72 (d, *J* = 8.4 Hz, 4H), 7.48 (d, *J* = 8.5 Hz, 4H), 4.44 (s, 4H); ^{13}C NMR (75 MHz, DMSO) δ 182.00, 137.84, 132.72, 132.30, 131.77, 128.34, 123.56, 43.77; EIMS m/z: 431(M-HCl); HREIMS Calcd. for $C_{19}H_{15}O_1N_1Br_2$: 430.9515; Found: 430.9509; Calcd. for $C_{19}H_{15}O_1N_1Br_1{}^{81}Br_1$: 432.9494; Found: 432.9493; Calcd. for $C_{19}H_{15}O_1N_1{}^{81}Br_2$: 434.9474; Found: 434.9479.

3) **p-CF3**

4-(Trifluoromethyl)benzaldehyde (2.61g,15mM) was used to synthesize instead of 2-Chlorobenzaldehyde. The method and the procession were the same as 3.2.1 description. Yield 70%; ^{1}H NMR (300 MHz, DMSO) δ 10.02 (s, 2H), 7.93 (s, 2H), 7.87 (d, *J* = 8.3 Hz, 4H), 7.74 (d, *J* = 8.4 Hz, 4H), 4.48 (s, 4H); ^{13}C NMR(75 MHz, DMSO) δ 182.07, 137.55, 137.42, 130.87, 129.81, 129.64, 129.22, 125.55, 122.02, 43.65; EIMS m/z: 411 (M-HCl). HREIMS Calcd. for $C_{21}H_{15}O_1N_1F_6$: 411.1052; Found: 411.1047.

2.3 Enzyme Activity Assay

The oxidation of CORT by 11β-HSD2(CORT represents cortisol in human) was determined in a mixture containing 0.2-0.5 μM CORT (plus 30,000 cpm [^{3}H]-CORT), 0.2 mM NAD^+, 10 mM DTT and 2% ethanol in 0.1mM potassium phosphate buffer (pH 7.2, 250 μL total volume) at 37 ℃ for 60 min. Because 11β-HSD2 in kidney microsomes is present as a dimer, 10 mM DTT (a reducing agent) was added to reaction buffer to disrupt kidney microsomes and the disulfyl group of 11β-HSD2 to convert 11β-HSD2 to as a monomer to increase it activity[17]. Reactions were initiated by addition of kidney microsomes (20 μg microsomal protein in human, NAD^+ and terminated by the addition of 2 ml ice-cold ether. The steroids were extracted by vigorous mixing for 1 min, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (90:10, v/v), and the radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of CORT to 11DHC and was calculated by dividing the radioactive counts identified as 11DHC by the total counts associated with CORT plus 11DHC as previously described[16], and the velocity of the 11β-HSD2 was calculated according to the percentage of substrate conversion, substrate concentration, enzyme amount and reaction time. The formation of product was determined at 4 time points within the linear portion of the reaction.

2.4 Enzyme Inhibition Studies

Inhibition of the oxidation of CORT catalyzed by 11β-HSD2 was measured using varying concentrations of each inhibitor with the substrate concentrations set to about 2 × *Km* to calculate the half maximal inhibitory concentration values (IC_{50}). The mode of inhibition was assayed by adding different fixed CORT concentrations in the presence of various concentrations of each inhibitor. Initial velocity data were fit to competitive, noncompetitive, uncompetitive, and mixed inhibition modes. The inhibition constant *Ki* was determined.

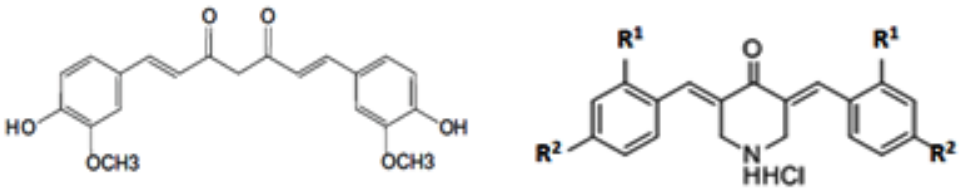
2.5 Statistics

Each experiment was repeated two to four times. Data were subjected to nonlinear analysis by GraphPad (Version 5, GraphPad Software Inc., San Diego, CA) for IC_{50}. Data were subjected to analysis by one-way ANOVA followed by DUNCAN multiple comparison testing to identify significant differences between groups when three and more groups were calculated. All data are expressed as means ± SEM. Differences were regarded as significant at $P < 0.05$.

3. Results

3.1 Synthesis of Novel Derivatives of Curcumin

The Chemical structures and its derivatives of three novel derivatives of curcumin (**o-Cl**, **p-Br** and **p-CF3**) were shown in Fig 2.Three compounds were added different R1 and R2,then the molecular weight and EI molecular weight were measured. The data were shown as follows.



Curcumin Compounds

Compounds	R^1	R^2	Molecular Weight (Da)	EI Molecular Weight(Da)
o-Cl	H	Cl	379	343
p-Br	Br	H	467	431
p-CF3	CF_3	H	447	411

Fig 2. Chemical structures of curcumin and its derivatives

3.2 The Result of Enzyme Activity Assay

We examined the 11β-HSD2 activity by different chemicals of derivatives of curcumin at 100 μM. The conversion of three synthesized curcumin derivatives were reduced obviously. The lowest conversion potency was **o-Cl**,which was only 6%±0.3%.The conversion of **p-Br** and **p-CF3** were 16%±0.8% and 22%±1.1%.(Fig.3)

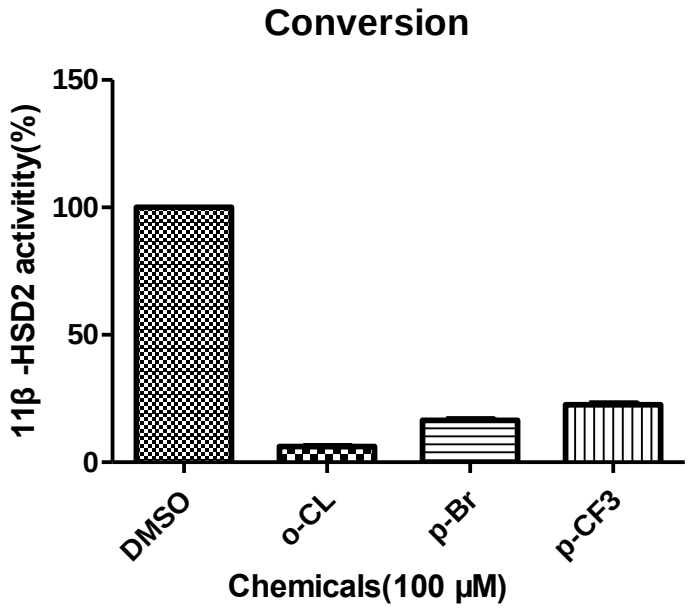


Fig 3.The conversion of different chemicals

3.3 The Result of Enzyme Inhibition Studies

We examined the inhibition of human 11β-HSD2 by a series of derivatives of curcumin at 100 μM. The potencies of inhibiting 11β-HSD2 human kidney microsomes for curcumin were 34.5 ± 1.7, (mean ± SEM, n=2) respectively. The inhibition of three synthesized curcumin derivatives were

shown in Table 1. For the inhibition of human 11β-HSD2, all synthesized chemicals were more potent than parent chemical curcumin. The highest inhibitive potency was **o-Cl**, which IC_{50} was approximately 430 times lower than that of curcumin. The reasons that the species-dependent difference for the potencies of inhibition of the enzyme were still unknown.

Table 1. The inhibition of curcumin derivatives on 11β-hydroxysteroid dehydrogenase type 2 in human kidney microsome

Compounds	IC50
o-Cl	0.08 ±0.004
p-Br	0.34 ±0.017
p-CF3	1.64 ±0.082
Curcumin	34.5 ±1.7

4. Discussion

Colorectal cancer, a hyper-proliferative disorder disease, accounts for 10% of all cancer deaths in the United States annually. It has been documented that up-regulation of cyclooxygenase-2 (COX-2) expression and the abundance of its enzymatic product prostaglandin E_2 (PGE_2) have key roles in the maintenance and progression of colorectal cancer. Further investigation indicated that inhibition of 11β-HSD2 could selectively block the tumor COX-2 activity[7]. Therefore, the selective 11β-HSD2 inhibitors will be touted as promising agents for chemoprevention and chemotherapy of CRC.

Curcumin has been used as safe therapeutic agent for colorectal cancer. According to the results of several clinical trials, it could decrease proliferation of various cancer cells, especially in the colon by selectively inhibiting the expression of COX-2[11, 12]. To improve the therapeutic effect of colorectal cancer, in this present study, we synthesized three novel derivates of curcumin and evaluated their inhibiting activity of 11β-HSD2 using kidney microsomes. There was no significant difference between these compounds in inhibiting the activity of 11β-HSD2 in human microsomes, but higher than curcumin.

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