Design, synthesis and activity of flavonoids aromatase inhibitors

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Abstract. Steroidal and non-steroidal aromatase inhibitors target the suppression of estrogen biosynthesis in the treatment of breast cancer. However, in this treatment regime, steroid-type inhibitors may have serious side effects. Therefore, in recent years, researchers have increasingly focused on developing non-steroidal derivatives and undertaken experiments to investigate the potential clinical use of these derivatives. As a kind of non-steroidal inhibitor of natural products, flavonoids have great potential to inhibit the activity of aromatic enzymes. In this study, 10 flavonoids-non-steroidal aromatase inhibitors were synthesized by alkylation reaction and structures were determined by 1HNMR analysis. The inhibitory activity of aromatase was determined by using CYP19/MFC high-throughput screening kit. Then, the effect of substituent on the inhibition activity of aromatic enzymes was summarized. The study could provide basic data and ideas for further searching for a higher activity of flavonoid aromatase inhibitors.

1. Introduction

Breast cancer is one of the most common cancer types. Surgical methods, radiotherapy, and reversible inhibition of estrogen synthesis or its interaction with drugs constitute the basis of treatment for breast cancer. The investigated history of breast cancer suggests that many tumors occur and develop through estrogen-dependent mechanisms. Aromatase (aromatase, CYP19) belongs to a compound enzyme in the family of cytochrome P 450[1, 2], which is a speed limiting enzyme responsible for the biotransformation of estrogen in the body. It can catalyze androgens to generate estrogen. Existing studies have shown that the function and expression of aromatase can significantly change the production of estrogen and interfere with local or systemic estrogen levels[3]. Therefore, the regulation of estrogen in vivo synthesis process by inhibiting aromatase activity has become another effective way to treat breast cancer. Currently, inhibitors of aromatase, one of the most commonly used drugs for breast cancer, have developed into the third generation[4]. Flavonoids, with high efficiency, low toxicity and wide biological activity, are the most widely distributed natural product-type non-steroidal aromatase inhibitors in plants. It has been reported that flavonoids, isoflavones and dihydroflavones have the effect of inhibiting aromatase[5]. A recent study showed that hesperidin inhibited the expression of aromatase in MCF-7 breast cancer cells at low concentrations. In addition, 5 isoflavones extracted from the Flemingia root in the Philippines showed a reversible competition to inhibit aromatase[6]. In this paper, 10 nuclei of 2-bit and 3-substituted flavonoids were synthesized by

alkylation reaction and the structural characterization was performed by 1HNMR analysis. Subsequently, it was confirmed that the new compounds have good aromatase inhibition activity by measuring their aromatase inhibitory activity, thus providing a new possibility for the treatment of breast cancer disease.

2.   Experimental section

2.1 General

All the chemicals used in the synthesis and biological activity analysis were purchased from Energy Chemical. 1 H NMR, Bruker 500 MHz UltraShield NMRSpectrophotometer (Bruker Corp, Billerica, Massachusetts, USA).

General procedure for the synthesis of flavonoid aromatase inhibitors （1-10）
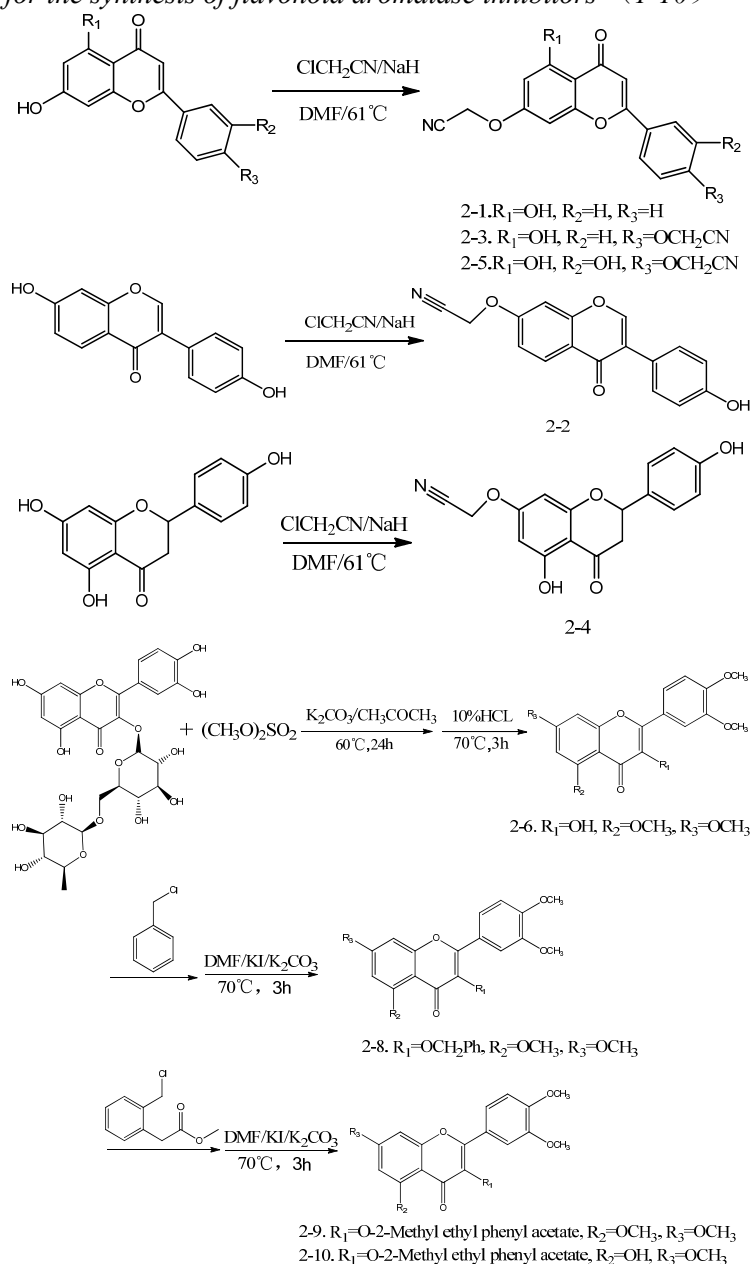

Fig. 1 Synthesis route of compound 1-10

In the synthesis process, the compounds 1-5 were all labelled with A (1 mmol) of the 50 mL round bottom flask, adding N, N' -dimethylformamide (DMF, 3 mL) and stirred for 5 minutes at room temperature. The sodium hydrogenated sodium (0.0480 g, 2 mmol) was added to the reaction fluid and continued to stir until it was no longer full. Chloracetonitrile was added at room temperature (503μ L, 4 mmol), and then added chloroacetonitrile after 30 min (76 μL, 1 mmol). The reaction bottle continue to be stirred in 61 ℃ oil bath, real-time monitoring reaction through TCL. After the reaction, the fluid was transferred to 500ml round - bottomed flask, add distilled water 300 mL and stir with glass rod. The ethyl acetate was then extracted and washed with water and salt water for several times. In addition, magnesium sulfate anhydrous was added to dry and the decompression rotation was obtained by crude product. In the end, the crude products were purified by silica gel column chromatography.

The synthesis process of compound 6

Accurately weigh 6g (9.828 mmoL) of rutin and 20 g (144.7 mmoL) of K2CO3 and place them in a dry 500 mL round bottom flask. To better dissolve the reactants, 15 mL of acetone added to the reaction solution. Then stir in 30 tube dimethyl sulfate in 60 ℃. Add the dimethyl sulfate 5 ~ 10 tubes to the reaction fluid at regular intervals and add twice. When the reaction goes to 12 h, we add 10 tubes of dimethyl sulfate, and we continue to react 12 h. After the reaction, add ammonia until it is no longer boiling. After the above reaction mixture was dried, the liquid was extracted with dichloromethane, and then the extractant was collected and then added 10% HCl solution to dissolve. Processing after 70℃ reflux flocculent solid 3h. The crude product is obtained by filtration. In the end, the crude products were purified by silica gel column chromatography.

The synthesis process of compound 7

Accurately weigh 6g (9.828 mmoL) of rutin and 20 g (144.7 mmoL) of K2CO3 and place them in a dry 500 mL round bottom flask. Add 10% HCl solution to dissolve, the reaction system get flocculent solid return 3 h at 70 ℃, the suction filter products. To better dissolve the reactants, 15 mL of acetone added to the reaction solution. Then stir in 30 tube dimethyl sulfate in 60 ℃. Add the dimethyl sulfate 5 ~10 tubes to the reaction fluid at regular intervals and add twice. Stop heating when the reaction goes to 12 h, adding suitable amount of NaH powder and dimethyl sulfate 10 tube after the temperature down to room lever, then heat up to 60 ℃ for 12 h. After the above reaction mixture was dried, the liquid was extracted with dichloromethane, and then the extractat was collected and then added 10% HCl solution to dissolve. The reaction system heats the backflow 7h under 30 ℃. The crude product is obtained by filtration. In the end, the crude products were purified by silica gel column chromatography.

The synthesis process of compound 8

The total methylated quercetin(0.500g) and 1.000 g (7.235 mmoL) K2CO3 were accurately weighed and placed in a dry 50 mL round bottom flask. With a small amount of KI as catalyst, the system to add 3 mL DMF and 3 tube of benzyl chloride, under the condition of 70 ℃ heating reflux 3 h. After the reaction, the reaction mixture was transferred to 250 mL separatory funnel and 100 mL water and a proper amount of dichloromethane were extracted. The crude products were purified by silica gel column chromatography.

The synthesis process of compound 9

The isochromone (5 g, 33.75 mmoL) was placed in a dry 250 mL round bottom flask. After 13ml methanol was added to the system, 15ml dichloromethane was added slowly under ice bath conditions. Stir for 2 hours. The pH>6 was adjusted with 10% K2CO3 solution, and ethyl acetate was added to the organic layer, and then the magnesium sulfate was added to the night.

The crude samples of total methylated quercetin (0.500 g) and 0.6800 g were measured. We add 1.000 g K2co3 and 7.235 g (3.012mmol) KI to the reaction bottle, make its solid powder dissolved in 3 mL DMF, 70 ℃ under the condition of stirring reflow 3 h, track to react completely with TLC. The post-treatment process is the same as compound 8.

The synthesis process of compound 10

Weighing the raw completely methylated quercetin (0.500 g), 2 - chloro methyl benzene acetic acid ethyl ester (0.6800 g, 4.246 tendency) and K2CO3 (1.000 g, 7.238 tendency), placing them in 250 ml of dry round bottom flask. To the system to add 0.05 g (3.012mmol) of KI and 3 mL DMF, return 3 h under the condition of 70 ℃, the track to react completely with TLC. The post-treatment process is the same as compound 8.

Aromatase inhibitory activity assay

The enzyme inhibition activity of the compound was determined by CYP19/MFC high-throughput screening kit, which was determined according to the established procedure.

3.   Results

3.1 Chemistry

1H NMR (400 MHz, Acetone-d6) δ 12.94 (s, 1H), 8.08 (d, J=7.1 Hz, 2H), 7.61 (d, J=7.6 Hz, 3H), 7.08~6.73 (m, 2H), 6.50 (d, J=2.1 Hz, 1H), 5.28 (s, 2H).

1H NMR (400 MHz, DMSO-d6) δ 9.75 (s, 1H), 8.43 (s, 1H), 8.10 (d, *J*=8.9 Hz, 1H), 7.39 (t, *J*=5.9 Hz, 3H), 7.20 (dd, *J*=8.9, 2.5 Hz, 1H), 6.85 (d, *J*=8.6 Hz, 2H), 5.39 (s, 2H).

1H NMR (400 MHz, DMSO-d6) δ 12.94 (s, 1H), 8.16 (d, *J*=8.9 Hz, 2H), 7.30 (d, *J*=9.0 Hz, 2H), 7.06 (s, 1H), 7.00 (d, *J*=2.3 Hz, 1H), 6.57 (d, *J*=2.3 Hz, 1H), 5.35 (d, *J*=1.5 Hz, 4H).

1H NMR (400 MHz, DMSO-d6) δ 12.09 (s, 1H), 9.61 (s, 1H), 7.33 (s, 2H), 6.80 (d, *J*=8.6 Hz, 2H), 6.25 (d, *J*=11.2 Hz, 2H), 5.54 (dd, *J*=13.0, 2.8 Hz, 1H), 5.24 (s, 2H), 3.50~3.28 (dd, 1H), 2.75 (dd, *J*=17.2, 3.0 Hz, 1H).

1H NMR (400 MHz, Acetone-d6) δ 12.38 (s, 1H), 10.46 (s, 1H), 7.43 (d, *J*=1.7 Hz, 2H), 6.95 (d, *J*=8.5 Hz, 1H), 6.62 (s, 1H), 6.12 (d, *J*=2.0 Hz, 1H), 5.79 (d, *J*=2.0 Hz, 1H), 4.90 (d, *J*=10.3 Hz, 4H).

^{1}H NMR (400 MHz, DMSO-d_6) 7.81 (d, *J*=8.6 Hz, 1H), 7.77 (s, 1H), 7.13 (d, *J*=8.6 Hz, 1H), 6.86 (s, 1H), 6.48 (s, 1H), 3.91 (s, 3H), 3.85 (s, 9H).

^{1}H NMR (400 MHz, DMSO-d_6) 7.81 (d, *J*=8.6 Hz, 2H), 7.77 (s, 1H), 7.13 (d, *J*=8.6 Hz, 1H), 6.86 (s, 1H), 6.48 (s, 1H), 3.91 (s, 3H), 3.85 (d, *J*=9.1 Hz, 12H).

1H NMR (400 MHz, DMSO-d6) 7.624~7.610 (d, *J*=8.6 Hz, 2H), 7.420 (s, 1H), 7.404 (s, 1H), 7.344~7.326 (m, 3H), 7.086~7.064 (d, *J*=8.6 Hz, 1H), 6.797~6.792 (d, *J*=8.2 Hz, 1H), 6.458~6.481 (d, *J*=8.2 Hz, 1H), 5.001 (s, 2H), 3.897 (s, 3H), 3.846 (s, 3H), 3.832 (s, 3H), 3.623 (s, 3H).

1H NMR (400 MHz, DMSO-d6) 7.55 (d, *J*=7.2 Hz, 1H), 7.24 (d, *J*=8.6 Hz, 1H), 7.20 (s, 1H), 7.07 (s, 1H), 7.03 (s, 1H), 6.81 (d, *J*=7.8 Hz, 1H), 6.52 (s, 1H), 6.29 (d, *J*=6.8 Hz, 1H), 5.90 (s, 2H), 4.35 (s, 2H), 4.08 (m, *J*=8.2 Hz, 2H), 3.91 (d, *J*=5.4 Hz, 1H), 3.83 (s, 1H), 3.79 (d, *J*=7.8 Hz, 6H), 3.68 (d, *J*=4.6 Hz, 1H), 3.17 (d, *J*=4.8 Hz, 3H).

^{1}H NMR (400 MHz, DMSO-d_6) 7.55 (d, *J*=7.2 Hz, 1H), 7.24 (d, *J*=8.6 Hz, 1H), 7.20 (s, 1H), 7.07 (s, 1H), 7.03 (s, 1H), 6.81 (d, *J*=7.8 Hz, 1H), 6.52 (s, 1H), 6.29 (d, *J*=6.8 Hz, 1H), 5.90 (s, 2H), 5.05 (s, 1H), 4.35 (s, 2H), 4.08 (m, *J*=8.2 Hz, 2H), 3.91 (d, *J*=5.4 Hz, 1H), 3.83 (s, 1H), 3.79 (d, *J*=6.8 Hz, 3H), 3.68 (d, *J*=5.6 Hz, 1H), 3.17 (d, *J*=4.8 Hz, 3H).

3.2 Aromatase inhibitory activity assay

Triazole was used as a positive control to test the synthesis of 10 compounds.The activity test is shown in table 1.

Tab. 1 Aromatase inhibition activity of the target compounds

Compound	IC50/(μmol·L-1)	Compound	IC50/(μmol·L-1)
1	0.36±0.09	7	18.60±0.13
2	0.46±0.14	8	2.73±0.17
3	0.76±0.17	9	0.75±0.11
4	0.86±0.08	10	1.651±0.09

| 5 | 0.56±0.07 | letrozole | 2.18±0.10 |
| 6 | 0.251±0.02 | | |

The inhibitory activity of the aromatase inhibitor showed that the substitution of different functional groups on the mother nucleus of flavonoids could affect the inhibitory activity of the aromatase inhibitor.

Compound 1 and 3-5 were both substituted for mother nuclei, with the same basic skeleton and different activity. Among them, the aromatase inhibitory activity of compound 1 was the most active when there was no substituent on the 2-bit substituted benzene ring. The activity was greatly reduced when the 2 substituted benzodiazepines were replaced by hydroxyl groups (compound 4).When hydrogen on 4-' hydroxyl group was replaced by -ch2cn (compound 3), the activity increased slightly. On the basis of the substitution of hydroxyl hydroxyl on the basis of -ch2cn, the activity of 3 'hydroxyl substituted (compound 5) was higher than that of compound 4, but it was still less than that of the benzene ring replaced by 2 bits. It is indicated that the substitution of 3 'and 4' bits on the substituted benzene ring can have a certain effect on the activity of the compound aromatase. Compounds 2 and 4 respectively, as a mother of three and two with hydroxy phenyl replace, from table 1 aromatase inhibition activity we can conclude that three of hydroxy phenyl replace the parent nucleus of aromatase inhibition activity is far greater than 2.

Although the basic skeleton of compound 6 and 8-10 are the same, compound 6 contains no hydroxyl substituents and the activity of aromatase is the best, while the other three compounds are slightly less active.

4. Conclusion
In general, the above results indicate that:

The aromatase activity of flavonoids was better than that of flavonoids when they were substituted by benzene ring. When two - bit substituted benzene rings were introduced, the activity of aromatase was decreased. The aromatase activity of the compound was the highest when there was hydroxyl substitution in 3 positions of the parent nucleus, and the aromatase inhibition activity of the compound was decreased when 3 positions of hydroxyl group were connected with the electron-donating group. This study provides basic data and ideas for the discovery of new flavonoid aromatase inhibitors with higher activity.

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