Research Article Open Access

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Synthesis and Activity of New Schiff Bases of Furocoumarin

https://doi.org/10.1515/hc-2020-0115
Received August 12, 2020; accepted November 04, 2020.

Abstract: Furocoumarins, such as 8-MOP, are the most common medications used to relieve the symptoms of vitiligo clinically. Some furocoumarins also showed excellent performance in an anti-bacterial assay. This paper describes the synthesis of a series of novel Schiff bases (**6a**-**6k**), and their promotion in melanogenesis and anti-bacterial properties were studied *in vitro*.

The pigment production of B16 cells and bacterial inhibition ring assay were applied for the bioactivity of **6a**-**6k**. According to the results, a stronger promotion on pigment content was observed, when six compounds co-cultured with cells, compared with positive control (8-MOP). Significantly, compound **6k** (237%) as the most active was found to increase the amount of melanin more than 1.7 times compared with 8-MOP activation rate (136%). All the compounds could moderately retard *C. albicans* growth. Interestingly, aldehyde **5**, which possessed a broader anti-bacterial spectrum, showed the highest inhibition against *C. albicans* as well and much better than the positive control (Amphotericin B).

Studies of **6k** in animal models of vitiligo and related molecular mechanism are presently under way, with the aim of discovering an anti-vitiligo leading compound.

Keywords: vitiligo, furocoumarin, melanin synthesis, anti-bacterial activity, SAR

Introduction

Vitiligo clinical features are irregular white spots of skin and mucous membrane often accompanied by gray-white hair, with the typical acquired cutaneous abnormalities [1,2] (**Figure 1**). It affects 50 million patients worldwide [3]. The disease can affect people of any gender or ethnic group and it is more likely to start during adolescence [4]. So far the mechanism of disease is still unclear, and people are much more likely to believe the autoimmune and inflammatory theory after considerable research.

The melanin pigments in skin, which was synthesized in human melanosomes, play an important role in the prevention of UV rays [6]. There are lots of factors (enzyme, cytokines, protein kinases) which may regulate this process through various signal pathways, such as tyrosinase (TYR), α-MSH (α-melanocyte stimulating hormone), MITF (microphthalmia-associated transcription factor), MAPK (mitogen-activated protein kinase), etc [7-9].

Current clinical drugs for vitiligo include furocoumarins, prednisone, tacrolimus, calcipotriol, khellin and, more recently, ruxolitinib [10,11]. As photosensitizers [12], derivatives of furocoumarin (**Figure 2**) were always orally or topically dosed and then combined with the UVB [13]. It was proved in many clinical cases that the furocoumarins were the most efficacious and led to cure or alleviate the vitiligo, although some side-effects were reported [14,15].

At present, there is little comprehensive and systematic research for new anti-vitiligo drug discovery. Our team

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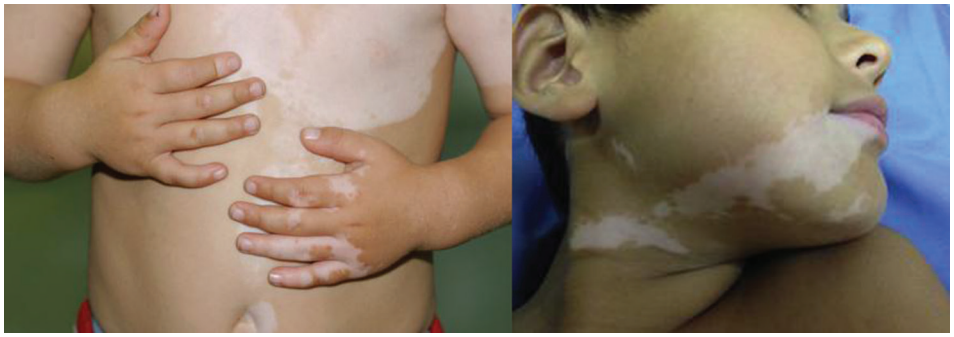


Figure 1 The main symptoms of vitiligo [1,2]

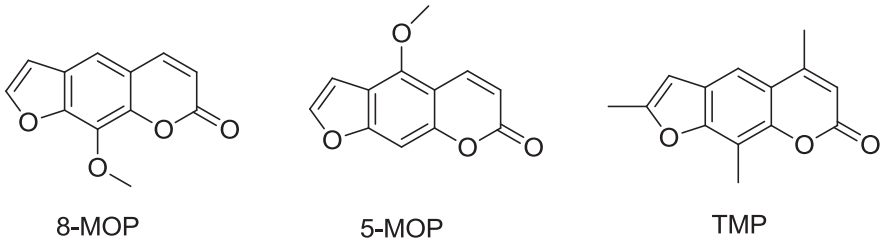


Figure 2 The clinically used furocoumarins for vitiligo

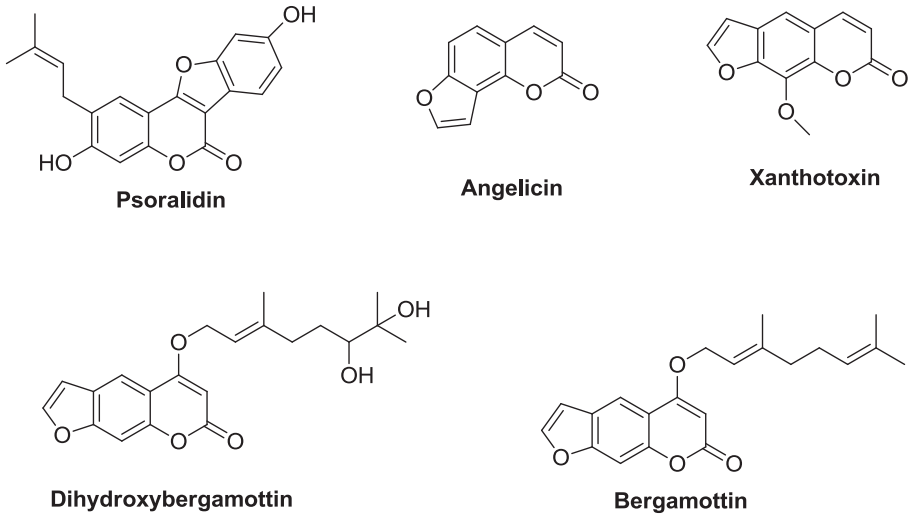
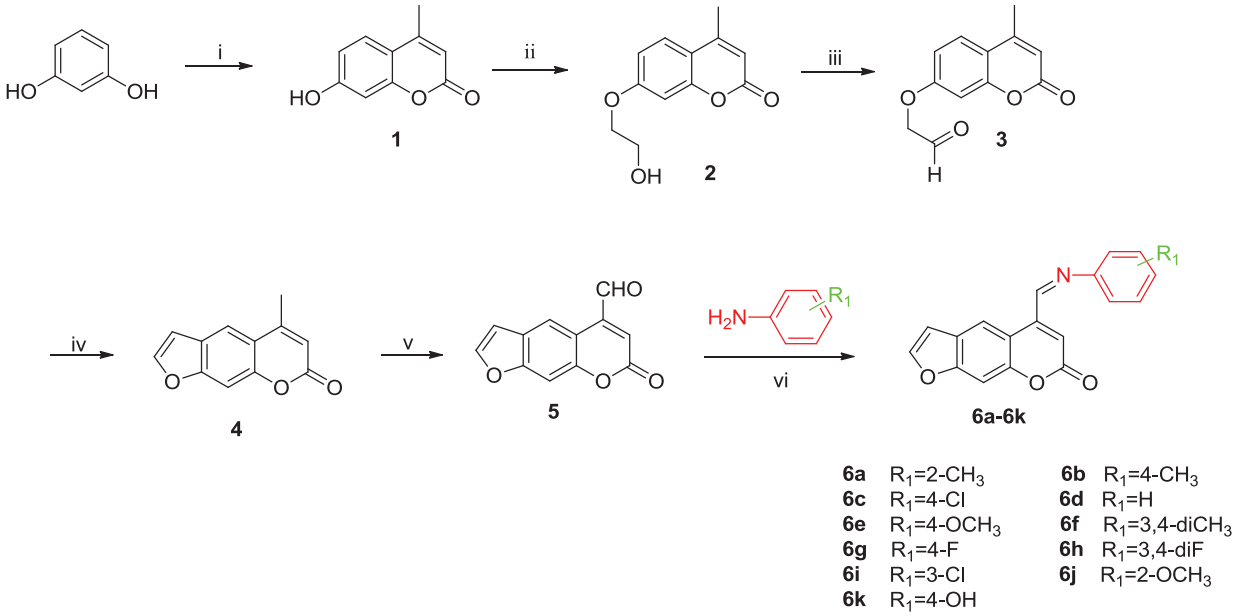


Figure 3 Structure of furocoumarins with anti-bacterial activity

are dedicated to search for leading compounds, which possess excellent potential development, from nature or synthesis over the years [16-22]. Based on previous experiences, introduction of substituted phenyl to C-5 provided us an approach of discovering new active molecules. We next modified the structure by constructing imineswith different anilines.

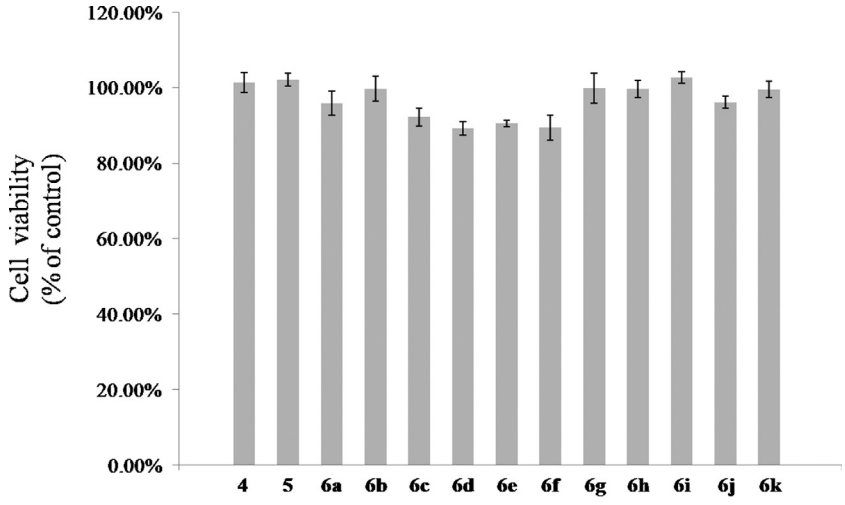
In addition, naturally occurring and synthetic furocoumarins were demonstrated to show anti-bacterial and anti-fungal activity [23-25], since they may act as autoinducer inhibitors targeting microbial cell signaling processes [26] (**Figure 3**).

In this research, eleven derivatives of Schiff bases (**6a**-**6k**) were prepared, aiming to exploit new leadsas anti-vitiligo drugs, and potential anti-bacterial agents that had wide spectrum and high-performance. We then evaluated these compounds for the enhancing effect on melanin synthesis at cell level, and bacteriostatic action on three different strains. After that, preliminary SAR was summarized as well.



Reagents and conditions: (i) ethyl acetoacetate, H_2SO_4, 60 °C; (ii) chloroethanol, K_2CO_3, acetone, reflux; (iii) -78 °C, oxalyl chloride, DMSO, triethylamine, DCM; (iv) 1M NaOH aqueous solution, 1,4-dioxane; (v) SeO2, xylene, reflux; (vi) ethanol, reflux

Scheme 1 Synthesis for Schiff bases **6a-6k**



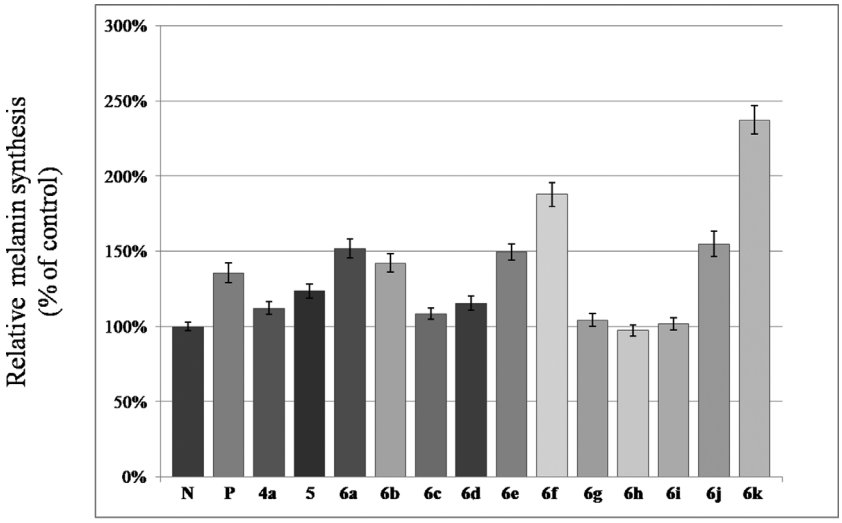
The B16 cells were incubated with 50 μM of different 6a-6k for 24 h and the cell viability was assayed byadding CCK-8 solution. Values are expressed as the mean ± SD of three separate experiments, P<0.05.

Figure 4 Cytotoxicity of Schiff bases **6a-6k**

Result and discussion

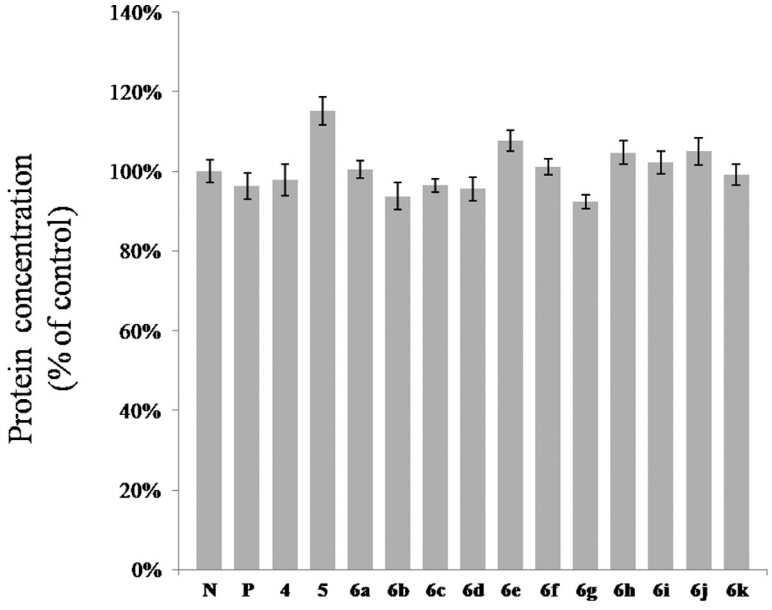
A route to synthesize **6a-6k** was illustrated below (**Scheme 1**). Resorcin was treated with ethyl acetoacetate in H_2SO_4 to yield 4-methylumbelliferone (**1**) [27].The successive hydrocarbylation performed under existence of chloroethanol and anhydrous potassium carbonatein acetone gave **2**. After that, aldehyde **3** was obtained by an optimized Swern Oxidation using DMSO and $(COCl)_2$ at -78 °C with high yields. Cyclization of **3**, dissolving in a mixture of water and 1,4-dioxane, yielded compound **4** in 1M NaOH for 6 h.

Further oxidation of **4** with selenium dioxide led to furocoumarin **5**. The desired derivatives **6a-6k** were



N means negative control; P means positive control (8-MOP); The B16 cells was treated with 50 μM of **6a-6k** for 48 h. After that, melanin content was measured directly. Values are expressed as the mean ± SD of three separate experiments, P<0.05.

Figure 5 Stimulation of Schiff bases **6a-6k** on melanin synthesis



N means negative control; 8-MOP as positive control. The B16 cells were incubated with 50 μM of different 6a-6k for 48 h and the protein concentration was assayed by the method described below. Values are expressed as the mean ± SD of three separate experiments, P<0.05.

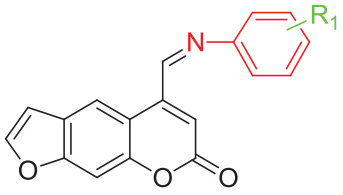
Figure 6 Cellular protein concentration of Schiff bases **6a-6k**

finally produced by refluxing **5** in 95% ethanol with corresponding aniline [28].

In vitro melanin synthesis evaluation

The CCK-8 assay was first performed to study the cytotoxicity of **6a-6k** to B16 cells, and none of the compounds showed toxic effects to the cells when the concentration was 50 μM (**Figure 4**). After that, the synthesized Schiff bases were evaluated for the ability to stimulate melanin synthesis in B16 cells [29] (**Figure 5**). Meanwhile, we measured protein concentration, in cells, to avoid errors. (**Figure 6**).

Compared to control group, more than half of synthesized compounds (**6a-6b**,**6e-6f**, **6j-6k**), of which activation rates were 142%-237%, had superior relative



6a-6k

Table 1 Anti-bacterial activity oftarget compounds **6a-6k**

Compounds	R_1	Concentration (mM)	Loading amount (μL)	Inhibition zone diameter (mm)		
				Candida albicans	*Escherichia coli*	*Staphylococcus aureus*
6a	2-CH_3	50	20	10	NI	NI
6b	4-CH_3	50	20	10	NI	9
6c	4-Cl	50	20	10	NI	11
6d	H	50	20	9	NI	NI
6e	4-OCH_3	50	20	10	NI	8
6f	3,4-diCH_3	50	20	10	NI	8
6g	4-F	50	20	12	NI	9
6h	3,4-diF	50	20	12	NI	16
6i	3-Cl	50	20	11	NI	NI
6j	2-OCH_3	50	20	10	NI	NI
6k	4-OH	50	20	10	NI	9
4	-	50	20	7	NI	NI
5	-	50	20	23	8	12
Ampicillin	-	3.0	20	-	14	NI
Ampicillin		0.3	20		NI	19
Amphotericin B	-	5.4	20	15	**NI**	**NI**

"NI": not inhibit growth of bacteria (inhibition zone diameters ≤7 mm).

melanin synthesis (**Figure 5**). It was clear that EDG (electron-donating group) substituted benzene (**6a-6b**, **6e**, **6j**), were beneficial to the effect, which was consistent with our previous findings [22]. Furthermore, an introduction of a second EDG could significantly enhanced the amount of melanin (**6f**>**6a**, **6b**). It was noticed that demethylation may remarkably enhance the stimulation effect as shown in **6k**, which was regarded as the most promising lead candidate. On the contrary, the activity of compounds substituted by -F (**6g**, **6h**) and -Cl (**6c**, **6i**) dropped dramatically irrelevantly to type, position and numbers of the halogens. In general, an EDG and hydroxy on benzene caused an increase in melanogenesis, and it was suspected that this maybe caused by electrostatic forces and hydrophilicity.

In vitro anti-bacterial activity evaluation

After that, all the Schiff bases were evaluated for their anti-bacterial efficiency against three species of bacteria, including *Candida albicans* (ATCC 10231, Fungi), *Escherichia coli* (ATCC 11229, Gram negative bacteria) and *Staphylococcus aureus* (ATCC 6538, Gram positive bacteria).

Table 1 showed that all the tested compounds could moderately inhibit the growth of *C. albicans* (*Candida albicans*). The introduction of an aldehyde on 5-position may significantly improve the inhibitory effect on *C. albicans* (**5**>**4**). Besides, compound **5**, which showed higher value than positive control (Amphotericin B) against *C. albicans*, possessed a broad spectrum of activity not only against *E. coli* (*Escherichia coli*) but also *S. aureus* (*Staphylococcus aureus*) as well.

Among **6a-6p**, compounds with any substituents (-F, -Cl, -CH_3, -OCH_3 or -OH) at *para*-position of benzene may generally inhibit the growth of the *S. aureus*(**6b-6c**, **6e-6h**, **6k**). And the halogen, including both -F and -Cl (**6c**, **6g**, **6h**), were more favorable to the activity than EDGs. Compound **6h** had comparable activity to the positive control on *C. albicans* and *S. aureus* which suggested that a second introduction of halogen to the benzene ring may strongly increase the inhibitory effect against *S.aureus*.

Conclusion

In this research, eleven novel 5-Schiff base substituted furocoumarin derivatives **6a**-**6k** have been synthesized based on our previous research. The melanin synthesis assay in B16 cells demonstrated that six compounds were better than control group. Furthermore, derivative **6k** (237%), which was 1.7-fold stronger than 8-MOP (136%), is now being studied for its mechanism of action *in vivo*.

In addition, all the Schiff bases were proved to inhibit the growth of *C. albicans in vitro*. Substitution at the *para*-position of benzene led to an increase in inhibition against *S. aureus*, and a halogen substituent seemed to be more preferable. It was impressive that compound **5**, which presented the strongest potency against *C. albicans*, had abroad-spectrum anti-bacterial activity against both *E. coli* and *S. aureus*. The proposed scaffolds of derivatives offer the possibility of convenient further modifications that could give rise to structures with improved anti-bacterial inhibitory activities.

Experimental section

All reagents were commercial and untreated. NMR were acquired with $CDCl_3$ and tetramethylsilane, which were solvent and internal standard, on Varian (400 and 600 MHz) spectrometers. Uncorrected melting points were measured using a Buchi B-540. Mass spectra were obtained using ABSciex QSTAR Elite quadrupole-time-of-flight mass spectrometry. Thermo Fisher Scientific Nilolet 6700 FT-IR infrared spectrometer was used to record infrared data.

Synthesis of 4-Methylumbelliferone (1)

The conc. H_2SO_4 (1.0 mL) was added slowly, to a stirring mixture of resorcinol (4.0 g, 36.4 mol) in dry 1,4-dioxaneunder ice bath, making it below 20 °C during the process. The solution was heated to 60 °C for 12 hours after an addition of ethyl acetoacetate (5.6 g, 42.0 mmol). When the starting material was consumed, the mixture was poured into water, a white solid **1** was obtained after filtrating and washing with methanol, Yield 89%, m.p. 200–203 °C.

Synthesis of7-(2-Hydroxyethoxy)-4-methyl-2H-chromen-2-one (2)

4-Methylumbelliferone (**1)** (0.44 g, 2.5 mmol) and chloroethanol (0.30 g, 3.8 mmol) were dissolved in dry acetone (30 mL), the mixture was heated to reflux for 6 h after which potassium carbonate (0.7 g, 5.0 mmol) was added. The solvent was evaporated and the residue was purified using flash column chromatography on silica gel to afford compound **2**. Yield 93%, white solid, m.p. 107–109°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J =9.0 Hz, 1H), 6.91–6.83 (m, 2H),6.15 (d, J =1.1 Hz, 1H), 4.15 (t, J =8.7 Hz, 2H), 4.01 (m, 2H), 2.40 (d, J =1.1 Hz, 3H).

Synthesis of7-(2-Oxoethoxy)-4-methyl-2H-chromen-2-one (3)

Oxalyl chloride (0.126 g, 1.0 mmol) was dissolved in dichloromethane (5 mL) at-78 °C filled with Ar. After addition of DMSO (0.078 g, 1.0 mmol), the mixture was stirred for 20 min until a dichloromethane solution of compound **2** (0.11 g, 0,05 mmol) was added dropwise. Half an hour later, Et_3N (0.250 g, 2.5 mmol) was added and the temperature was increased to 25 °C slowly. The solvent was then evaporated to yield compound **3**. Yield 95%, white solid, m.p. 142–144°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.53 (d, J = 8.8 Hz, 1H), 6.89 (dd, J = 8.8, 2.6 Hz, 1H), 6.78 (d, J = 2.5 Hz, 1H), 6.15 (d, J = 1.0 Hz, 1H), 4.68 (s, 2H), 2.39 (d, J = 0.9 Hz, 3H).

Synthesis of5-Methyl-7H-furo[3,2-g]chromen-7-onecompounds (4)

The compound **3** (0.22 g, 1.0 mmol), which dissolved in a mixed solvent of H_2O and 1,4-dioxane, was added to an aqueous solution of sodiumhydroxide, and then refluxed. The reaction was cooled until the disappearance of raw materials then extracted with AcOEt (3 x 50 mL) after neutralization. The organic extracts were concentrated and purified by silica-gel column chromatography to give **4** as a white solid. Yield 70%, m.p. 158–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.69 (d, J = 2.2 Hz, 1H), 7.47 (s, 1H), 6.85 (d, J = 2.1 Hz, 1H), 6.27 (s, 1H), 2.50 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 161.24, 156.43, 152.78, 151.75, 146.96, 124.77, 116.70, 113.62, 106.67, 100.00, 19.30;IR (KBr) *v*: 2921, 1723, 1630, 1385, 1137, 1031, 928 cm^{-1}; HRMS (ESI) calcd for $C_{12}H_9O_3[M+H]^+$ 201.0552, found 201.0540.

Synthesis of7-Oxo-7H-furo[3,2-g]chromene-5-carbaldehyde (5)

A solution of compound **4** (4.0 g, 10 mmol) and selenium dioxide (1.67 g, 15 mmol) in anhydrous xylene (50 mL) was

heated to 140 °C and stirred for 24 h. The Se was filtered and the resulting filtrate was evaporated. Compound **5** was acquired via column chromatography purification. Yield 63%, yellow solid, m.p. 176–177°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 8.90 (s, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.54 (s, 1H), 6.89 (d, J = 2.0 Hz, 1H), 6.86 (s, 1H).

General synthesis method of Schiff bases 6a–6k

A mixture of Compound **5** (1.0 mmol) and corresponding aniline (3.0 mmol) in ethanol (20 ml) was refluxed. After 24 h, the reaction was evaporated and purified by silica gel chromatography to furnish **6a**–**6k**.

(*Z*)-5-((2-methylphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6a). Yield 40%, light yellow solid, m.p. 181–183°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.23 (s,1H), 8.59 (s, 1H), 7.71 (d, J= 2.1 Hz, 1H), 7.55 (s, 1H), 7.34–7.26 (m, 3H), 7.03 (d, J= 7.2 Hz, 1H), 6.87 (d, J= 1.9 Hz, 1H), 6.79 (s, 1H), 2.46 (s, 3H). ^{13}C NMR (101MHz, $CDCl_3$): *δ* 161.19, 156.77, 156.38, 152.62, 149.92, 147.06, 146.14, 133.02,130.91, 127.79, 127.19, 125.17, 119.59, 118.71, 117.10, 113.41, 107.12, 100.20, 18.35. IR (KBr) v: 2924, 2853, 1709, 1634, 1448, 1156, 1134, 1012, 760 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{14}NO_3[M+H]^+$304.0968, found 304.0990.

(*Z*)-5-((4-Methylphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6b). Yield 38%, light yellow solid, m.p. 193–194°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.18 (s,1H), 8.67 (s, 1H), 7.70 (d, J= 2.2 Hz, 1H), 7.54 (s, 1H), 7.05 (d, J= 8.1 Hz, 2H), 6.90–6.85 (m, 3H), 6.77 (s, 1H), 2.42 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): *δ* 161.19,156.35, 152.60, 148.19, 147.01, 146.27, 138.29, 130.24, 125.14, 121.21, 119.60,118.35, 116.04, 113.42, 107.14, 100.17, 21.31; IR (KBr) v: 2926, 2851, 1707, 1630,1445, 1150, 1135, 1011, 944, 759 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{14}NO_3[M+H]^+$304.0968, found 304.0948.

(*Z*)-5-((4-Chlorophenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6c). Yield 31%, yellow solid, m.p. 200–202°C; ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 9.13 (s,1H),8.64 (s, 1H), 7.71 (d, J= 2.0 Hz, 1H), 7.55 (s, 1H), 7.45 (d, J= 7.4 Hz, 2H), 7.28 (d, J= 8.2 Hz, 2H), 6.89 (d, J= 1.9 Hz, 1H), 6.78 (s, 1H).^{13}C NMR (151 MHz, $CDCl_3$): *δ* 160.84, 157.72, 147.23, 146.99, 145.68, 138.10, 133.61, 129.65, 125.06, 124.42,122.36, 113.02, 119.28, 118.88, 106.96, 100.14. IR (KBr) v: 1717, 1623, 1576, 1234,1076 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{11}ClNO_3[M+H]^+$ 324.0422, found 324.0440.

(*Z*)-5-(Phenylimino)methyl-7H-furo[3,2-g]chromen-7-one (6d). Yield 36%, yellow solid, m.p. 210–211°C; ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 9.16 (s, 1H), 8.66 (s,1H), 7.71 (d, J= 2.1 Hz, 1H), 7.55 (s, 1H), 7.51–7.46 (m, 2H), 7.36 (t, J=7.4 Hz,1H), 7.33 (d, J= 7.4 Hz, 2H), 6.89 (d, *J* = 2.1 Hz, 1H), 6.78 (s, 1H). ^{13}C NMR(151 MHz, $CDCl_3$): *δ* 161.12, 157.54, 156.42, 152.59, 150.82, 147.06, 146.10, 129.63,127.94, 125.18,124.55, 121.12, 119.56, 118.72, 113.31, 107.13, 100.21. IR (KBr) v: 1705,1633, 1449, 1154, 1132, 761 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{12}NO_3[M+H]^+$290.0812, found 290.0798.

(*Z*)-5-((4-Methoxyphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6e). Yield 34%, light yellow solid, m.p. 188–190°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.18 (s,1H), 8.69 (s, 1H), 7.70 (d, J= 2.2 Hz, 1H), 7.53 (s, 1H), 7.39 (d, J= 8.9 Hz, 2H), 7.01(d, J= 8.9 Hz, 2H), 6.89 (d, J= 2.0 Hz, 1H), 6.76 (s, 1H), 3.88 (s, 3H). ^{13}C NMR(151 MHz, $CDCl_3$): *δ* 161.26, 160.05, 156.35, 154.52, 152.58, 146.98, 146.37, 143.46, 125.08,122.99, 119.55, 117.85, 116.80, 114.84, 113.50, 107.12, 100.14, 55.74. IR (KBr) v:2924, 2843, 1706, 1631, 1560, 1446, 1153, 759 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{14}NO_4[M+H]^+$ 320.0917, found 320.0940.

(*Z*)-5-((3,4-Dimethylphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (**6f**). Yield 33%, light yellow solid, m.p. 165–167°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.17 (s,1H), 8.66 (s, 1H), 7.70 (d, J= 2.2 Hz, 1H), 7.52 (s, 1H), 7.23 (d, J= 7.9 Hz, 1H), 7.16(s, 1H), 7.11 (dd, J= 7.9, 2.0 Hz, 1H), 6.89 (d, J= 2.1Hz, 1H), 6.74 (s, 1H), 2.34 (s,3H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): *δ* 161.19, 156.33, 156.08,152.56,148.46, 146.97, 146.29, 137.97, 136.96, 130.70, 125.09, 122.64, 119.61, 118.45,118.20, 113.41, 107.13, 100.10, 20.02, 19.65. IR (KBr) v: 2923, 2855, 1704, 1637,1568, 1447, 1157, 1130, 762 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{16}NO_3[M+H]^+$318.1125, found 318.1145.

(*Z*)-5-((4-Fluorophenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6g). Yield 30%, light yellow solid, m.p. 179–181°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.13 (s,1H), 8.65 (s, 1H), 7.71 (d, J= 2.2 Hz, 1H), 7.54 (s, 1H), 7.39–7.31 (m, 2H), 7.22–7.12(m, 2H), 6.89 (d, J= 1.9 Hz, 1H), 6.77 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): *δ* 161.23,157.07, 152.77, 151.77, 147.39, 146.97, 145.95, 125.17, 122.97, 119.44, 118.71,116.69, 116.40, 113.64, 107.10, 106.68, 100.03. IR (KBr) v: 1720, 1625, 1569, 1450,1228, 1082, 755 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{11}FNO_3[M+H]^+$308.0717, found 308.0693.

(*Z*)-5-((3,4-Difluorophenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6h). Yield 28%, light yellow solid, m.p. 163–164°C; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 9.09 (s, 1H), 8.62 (s, 1H), 7.69 (d, J= 2.2 Hz, 1H), 7.49 (s, 1H), 6.91–6.89 (m, 2H),6.87–6.84 (m, 2H), 6.79 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): *δ* 161.26, 156.47,154.30, 152.79, 147.39, 146.98, 145.23, 132.69, 127.82, 124.57, 119.15, 116.70,113.66, 110.18, 108.74, 106.68, 100.32. IR (KBr) v: 1722, 1630, 1569, 1457, 1334, 1231,1100, 763 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{10}F_2NO_3[M+H]^+$ 326.0623, found326.0651.

(*Z*)-5-((3-Chlorophenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6i). Yield 31%, yellow solid, m.p. 192–193°C; ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 9.12 (s,

1H),8.63 (s, 1H), 7.72 (d, J= 2.1 Hz, 1H), 7.56 (s, 1H), 7.45–7.38 (m, 1H), 7.36–7.31 (m,2H), 7.21 (d, J= 8.3, 1H), 6.90 (d, J= 2.0 Hz, 1H), 6.79 (s, 1H). ^{13}C NMR (101 MHz,$CDCl_3$): δ 161.31, 157.36, 154.20, 152.51, 150.13, 148.07, 147.41, 133.73, 131.18,128.41, 126.87, 125.38, 120.92, 118.62, 117.79, 114.73, 108.66, 101.11. IR (KBr) v:1717, 1635, 1580, 1461, 1227, 1076, 756 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{11}ClNO_3[M+H]^+$ 324.0422, found 324.0439.

(*Z*)-5-((2-Methoxyphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6j). Yield 35%, light yellow solid, m.p. 169–170°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.13 (s,1H), 8.76 (s, 1H), 7.69 (d, J= 2.2 Hz, 1H), 7.53 (s, 1H), 7.33–7.28 (m, 1H), 7.15 (dd,J= 7.9, 1.4 Hz, 1H), 7.08–7.01 (m, 2H), 6.87 (d, J= 2.1, 1H), 6.76 (s, 1H), 3.94 (s,3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.26, 156.44, 152.79, 151.77, 147.38,146.97, 124.78, 124.56, 121.24, 119.14, 116.70, 113.64, 107.11, 106.68, 100.30,100.04, 55.67. IR (KBr) v: 2925, 2850, 1706, 1635, 1576, 1444, 1154, 1136, 758 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{14}NO_4[M+H]^+$ 320.0917, found 320.0933.

(*Z*)-5-((4-Hydroxyphenyl)imino)methyl-7H-furo[3,2-g]chromen-7-one (6k). Yield 30%, yellow solid, m.p. 218–220°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.18 (s,1H),8.68 (s, 1H), 7.71 (d, J= 2.2 Hz, 1H), 7.54 (s, 1H), 7.35 (d, J= 8.6 Hz, 2H), 6.95 (d, J= 8.8 Hz, 2H), 6.89 (d, J= 2.0 Hz, 1H), 6.76 (s, 1H). ^{13}C NMR (101 MHz,$CDCl_3$): δ 163.43, 157.74, 155.91, 154.56, 148.87, 141.31, 138.07, 132.03, 124.53,122.85, 120.55, 116.92, 115.81, 111.55, 107.95, 100.97. IR (KBr) v: 3367, 1705, 1634,1448, 1152, 1133, 760 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{12}NO_4[M+H]^+$ 306.0761, found 306.0736.

Biological activity

Melanin contents assay

The inoculation of B16 cells, which was in the period of exponential growth, was conducted on 6-well plates, and keeping a concentration at 5×10^5 cells per well. The cells were cultured at 37 °C for 24 h before removal of the media. A mixture of different compounds in medium was added to the cells and allowed to grow for another 48 h. Washing with PBS, the cells were lysed at low temperature under the effect of RIPA buffer. After centrifugation of lysates at 12,000 for 15 min, the protein in the supernatant was analyzed, followed by dissolution of melanin within cells in sodium hydroxide (1 M) at 80 °C for 1 h. The melanin content was assessed via spectrophotometry at 405 nm, and calculated by normalization of total melanin value with protein content (abs melanin/μg protein).

Anti-bacterial activity assay

The agar spread method was employed to determine the anti-bacterial activity of **6a**-**6k**. Samples of the strain were incubated at 37 °C for 18 h after inoculation with a suitable medium. After centrifugation the strains were kept in suspension in sterile water. The well was produced by the mixture of bacteria (1 mL) and agar media (100 mL). After addition of compounds, the wells were cultured at 37 °C for 24 h. Inhibitory activity was evaluated, based on the measurement of the diameter of inhibition zone. Averages were obtained from three assays and the compound was considered as inactive if the diameter ≤7 mm.

Acknowledgements: This research was financially supported by the Xinjiang Key Laboratory of Xinjiang Indigenous Medicinal Plants Resource Utilization (No. 2018D04020); Funds for the Youth Innovation on Promotion Association, Chinese Academy of Science (2019425); Tianshan Youth Program of Xinjiang Autonomous Region in 2019 (2019Q028).

Conflict of interest: The authors state no conflict of interest.

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