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Synthesis of Pichromenes, a Potential Anticancer Agent Using Organocatalyst

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Abstract: Pichromene 1 is a potential anti-cancer substance that is being studied for treatment of chronic leukemia. In this paper have been developed method for synthesis of pichrmene 1 and its analogs by condensation between substituted salicylaldehyde and β-nitrostyrene derivatives under different conditions (solvent, reaction time, temperature and catalyst…). The research results showed that in toluene, at a temperature of 80^0C, with acid L-pipecolic as catalyst, the yield of pichromenes reached 82% after 24h.
The structure of the products was determined by the data of ^{1}H-NMR and ^{13}C-NMR spectroscopy.

1. Introduction

2H-chromenes, or 2H-1-benzopyrans are series of oxygen heterocycles that form common structural motifs in a various natural products. The 2H-chromene moiety is found in tannins and polyphenols which commonly present in fruits, vegetables, teas and red wines. Interest in these compounds is increasing because of their anti-tumour and anti-bacterial activity [1,2]. Although the last few years there have been published many methods for synthesis of chromenes [3-5], the search for new approach to its derivatives still intensively continued. Because there have been discovered novel compounds containing chromene structure, which possess high biological activity. One of these new agents is 8-ethoxy-2-(4-fluorophenyl)-3-nitro-2H-chromene (Pichromene 1, fugure 1), which is capable to inhibit expression of cyclins D1, D2, and D3 in myeloma and leukemia cell lines at low micromolar concentrations. Pichromene 1 can also arrest the cells in the G0/G1 phase of the cell cycle. Furthermore, in myeloma and leukemia cell lines, pichromene 1 decrease levels of phospho-AKT, but did not alter levels of total AKT [6].

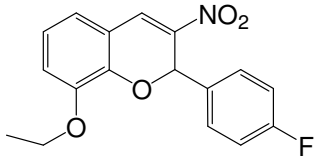

Fig. 1: Pichromene 1.

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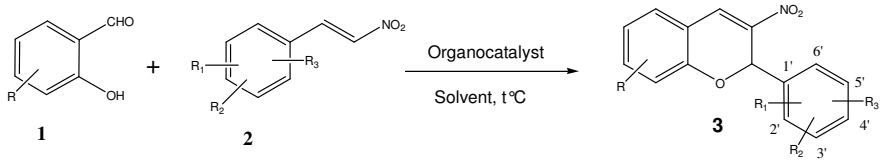
Since the moment of detecting this agent have been published some papers dealing with the synthesis and testing of its biological activity [6,7]. However, it is unknown the general approach to the synthesis, as well as the conditions of its performance. So there is still necessary comprehensive study of the process for further development of strategies to new substituted chromene systems.

In this paper, we report a systematic study for developing a simple, fast and cost-effective way to synthesize new pichromene 1 analogues.

2. Result and Discussion

Synthesis of pichromene 1 and analogues were based on oxa-Michael-Henry condensation of substituted salicylaldehyde with β-nitrostyrene derivatives, as described on Scheme 1.

Scheme 1



3a: R = R_1 = R_2 = R_3 = H; **3b**: R = 8-OEt, R_1 = R_2 = H, R_3 = 4'-Cl; **3c**: R = 8-OEt, R_1 = R_2 = H, R_3 = 4'-F;
3d: R = 8-OMe, R_1 = R_2 = H, R_3 = 4'-Cl; **3e:** R = 8-OMe, R_1 = 2'-Br, R_2 = 5'-F, R_3 = H;
3f: R = 8-OMe, R_1 = 3'-OMe, R_2 = 5'-OMe, = R_3 = 4'-OMe; **3g**: R = 7-OMe, R_1 = 2'-Br, R_2 = 5'-F, R_3 = H;
3h: R = 7-OMe, R_1 = R_2 = H, R_3 = 4'-Cl; **3i:** R = 6-Br, R_1 = 2'-Br, R_2 = 5'-F, R_3 = H

It was known from the literature, these reactions are stimulated by organocatalysts. Shakakibara et all [6] showed that, the condensation of unsubstituted salicyaldehyde with β-nitrostyrene in CCl_4 solvent and catalysed by Et_3N afforded chromene **3a** with the yield of 38%. Mao Xinliang et all [8] curried out reaction between 3-ethoxy-salicylaldehyde with some 4'-F-*β*-nitrostyrene in an excess of Pyridine used as both solvent and catalyst and obtained Pichromene 1 with the yield of about 30%. Obviously, the yields of targeted products in both of the above mentioned publications used Et3N and Pyridine as catalysts are unsatisfactory. Moreover the use of large amounts of pyridine [8] is harmful to the environment. In order to improve the yield of the desired pichromenes, we have attempted to examine influence of a wide range of catalysts (figure 2). The results of the investigation are described in the table 1.

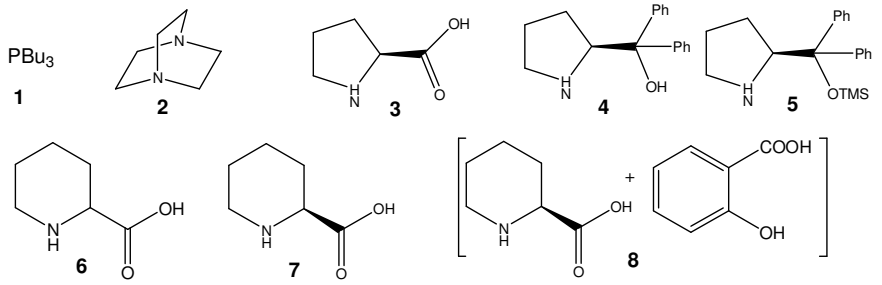


Fig. 2. Screened organicatalysts.

The research has started with tributylphosphine. In solvent DMF, at room temperature for three days (entry 1), the product of the reaction catalysed by tributyl-phosphine was not observed (checked by TLC). The attempt to improve the yield by increasing temperature up to 100^0C ended by a negative result.

Similarly to tributylphosphine, crude reaction mixture in the presence of DABCO (entry 2) did not show any characteristic signals of the product, that is, two singlet peak of proton at 6,64 ppm and 8,03 ppm in 1H-NMR spectrum. Therefore, we had to change the condition and use other catalysts.

L-proline is an amino acid which has a chiral center, making it a potential enantioselective catalyst. 20 mol % of this catalyst was used for the condensation reaction in DMF/water at room temperature for 3 days. In 1H-NMR spectrum of crude reaction mixture, a small amount of the desired product was generated. Pichromene 1 was readily purified by column chromatography and confirmed by 1H-NMR. The 1H-NMR spectrum of the pure pichromene clearly indicated two singlet at 6.64 ppm and 8.03 ppm corresponding to the set of proton at positions H-1 and H-2. In addition, the quartet, located at 4.03 ppm (2H, J=7.1 Hz) and the triplet at 1.36 ppm (3H, J=7.1 Hz), are signals of ethoxy group. Specifically, a doublet at 7.37 (1H, J=5.25 Hz) and 7.38 (1H, J=5.2 Hz) corresponds to the sets of protons at positions H-6 and H-9, a triplet at 6.99 ppm (1H, J=8.6 Hz) confirms the proton at H-4, and two doublets, located 6.93 and 6.95 ppm represent the two sets H-3 and H-5.

Table 1. Synthesis of Pirchromene 1 by using organocatalyst

Entry	Catalyst	Solvent	Temperature, ^{0}C	Time, h	Yield, %
1	1	DMF	rt	24 days	None
2	2	DMF	100	24 days	None
3	1	Toluene	80	24 hours	None
4	2	Toluene	80	24 hours	None
5	3	Toluene	80	24 hours	63%
6	4	Toluene	80	24 hours	9%
7	5	Toluene	80	24 days	14%
8	6	Toluene	80	24 hours	71%
9	7	Toluene	80	24 hours	82%
10	8	Toluene	80	24 hours	24%
11	6	DMF/water	rt	24 days	18%
12	7	DMF/water	100	24 days	62%
13	8	DMF/water	rt	24 days	None

However, the yield of reaction is very low. Thus, the condition of reaction was changed: dry toluene, argon atmosphere, 80^oC for 24 hours. Reaction yield was significantly improved (Entry 3).

Then, different catalysts were tested (entry 4 to 12); interestingly, this reaction proceeded quite well in the presence of amino acids such as pipecolic and L-pipecolic acid. Reaction conditions and yields have been showed on the table **1**.

Similar to L-proline 3, L-pipecolic acid **7** is an organocatalyst with high enantioselectivity. In the mechanism of reaction, organocatalyst

plays an important role. Firstly, pipecolic acid. which has a chrial center and one non-bonding electron pair, combines with aldehyde and lose a water molecule to establish iminium. Then, iminium combines with α,β-unsaturated compound by donating the non-bonding electron pair on hydroxyl group. The carbon, which belongs to imine part, accepts electrons from carbon-carbon double bond of α,β-unsaturated compound and donates the electron to nitrogen. The very acidic proton, which is adjacent to the nitro group, combines with the non-bonding electron pair on nitrogen to make a new N-H bond. After that, pipecolic acid is recovered and pichromene 1 is established.

Table 2. The Properties and IR data of pichromene1 and derivatives

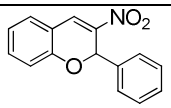
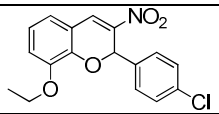
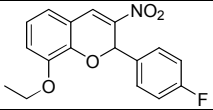
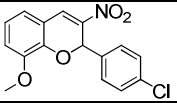
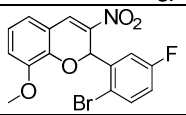
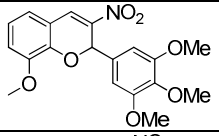
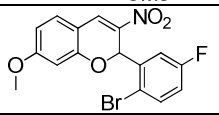
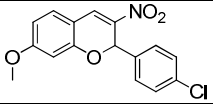
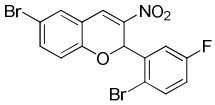
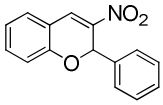
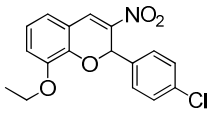
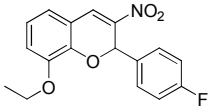
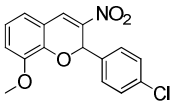
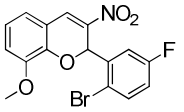
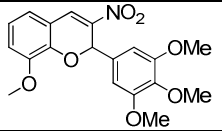
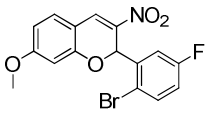
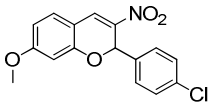
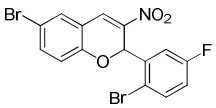
TT	CTCT	Yield, %	T. melt., ^{0}C	IR (cm^{-1})		
				ν_{NO2}	ν_{OR}	υ-aryl
1		70	90.1 – 92.8	1505, 1325	1246	1400-1600
2		70	114.5 – 115.4	-	-	
3		60	118.0 – 120.0	1504, 1318	1240	1400-1600
4		74	155.1 – 157.0	1506, 1319	1250	1400-1600
5		80	160.7 – 162.3	-	-	
6		53	152.3 – 154.1	1481, 1322	-1238	1400-1600
7		74	135.6 – 137.4	1497, 1305	1249	1400-1600
8		65	115.9 – 118.1	1495, 1304	1250	1400-1600
9		68	140.9 – 143.2	-	-	-

Table 3. ^{1}H-NMR of pichromene 1 and derivatives

TT	CTCT	^{1}H-NMR($CDCl_3$; δ, ppm; J,Hz)	^{13}C-NMR($CDCl_3$; δ, ppm)
1		8.07 (s, 1H), 7.41–7.31 (m, 7H), 7.05–6.99 (m, 1H), 6.88–6.86 (m, 1H), 6.60 ppm (s, 1H)	153.56, 141.19, 136.79, 134.31, 130.44, 129.48, 129.28, 128.85, 127.03, 122.54, 117.94, 117.29, 74.24
2		8.03 (s, 1H), 7.34–7.25 (m, 4H), 6.96–6.85 (m, 3H), 6.63 ppm (s, 1H), 4.08–3.97 (m, 2H), 1.40 (t, J=7.2Hz, 3H)	147.98, 142.89, 141.11, 135.25, 129.61, 128.94, 128.25, 122.68, 118.89, 118.59, 73.16, 65.01, 14.71
3		8.03 (s, 1H), 7.39–7.35 (m, 2H), 6.99–6.92 (m, 5H), 6.64 (s, 1H), 4.04–3.99 (q, J=6.9Hz, 2H), 1.36 (t, J=6.9Hz, 3H)	164.15, 162.18, 147.94, 142.92, 141.27, 132.58, 129.42, 128.81, 122.55, 122.19, 118.87, 115.75, 73.20, 65.05, 14.66
4		8.04 (s, 1H), 7.35–7.28 (m, 4H), 6.98–6.93 (m, 3H), 6.63 (s, 1H), 3.83 (s, 3H)	148.61, 142.37, 141.02, 135.36, 135.10, 129.43, 129.00, 128.30, 122.66, 122.07, 118.56, 116.75, 73.41, 56.26
5		8.16 (s, 1H), 7.66–7.62 (dd, J=8.7Hz and J=5.2Hz, 1H), 7.03–6.93 (m, 6H), 3.77 ppm (s, 3H, CH_3)	163.13, 160.66, 148.80, 142.19, 139.91, 136.64, 135.17, 130.50, 122.81, 122.28, 118.43, 118.23, 117.97, 115.67, 72.86, 56.58
6		8.05 (s, 1H), 6.98–6.95 (m, 3H), 6.62 (s, 3H), 3.86 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.77 (s, 6H, CH_3)	153.30, 146.62, 141.49, 138.75, 132.00, 129.20, 122.60, 121.96, 118.83, 116.57, 103.95, 74.02, 60.74, 56.23, 56.03
7		8.17 (s, 1H), 7.66 (dd, J = 8Hz and J = 4Hz, 1H), 7.30 (s, 1H), 6.97 (m, 3H), 6.61 (td, J=8Hz and J=4Hz, 1H), 6.38 (s, 1H), 3.79 (s, 3H, CH_3).	165.42, 163.23, 160.76, 154.90, 137.02, 135.18, 131.81, 130.94, 118.36, 118.13, 115.62, 115.38, 110.34, 102.31, 73.06, 55.69
8		8.04 (s, 1H), 7.29 – 7.22 (m, 5H), 6.58 (dd, J=8.5Hz and J=2.4Hz, 1H), 6.52 (s, 1H), 6.38 (d, J=2.7Hz, 1H), 3.78 (s, 3H, CH_3)	165.23, 155.32, 137.94, 135.51 135.32, 131.79, 129.96, 129.04, 128.41, 110.92, 109.95, 102.24, 73.73, 55.65
9		8.09 (s, 1H), 7.68 (dd, J=8.8Hz and J=5.4Hz, 1H), 7.51 (d, J=2.4Hz, 1H), 7.44 (dd, J=8.7Hz and J=2.3Hz, 1H) 7.00 – 6.95 (m, 2H), 6.88 (dd, J=8.8Hz and J=2.9Hz), 6.77 (d, J=8.8Hz, 1H)	163.19, 160.72, 151.75, 137.08, 136.26, 135.39, 132.47, 129.00, 119.20, 118.68, 118.47, 115.57, 115.33, 114.92, 72.98

According the results, we determined the best conditions for the synthesis of pichromene 1: catalyst L-pipecolinic acid **7,** solvent: toluene, temperature: 80^0C, argon atmosphere, reaction time: 24 hours. The pichromenes prepared by our method will be used for investigation of biological activities against leukemia in comparison with the previously reported Pichromene compounds.

3. Experimental

Synthesis of 4-fluoro-β-nitrostyrene

A beaker containing the mixture of *p*-fluorobenzaldehyde (11,6g, 95 mmol) and nitromethane (5.8g, 95 mmol) in 30mL of methanol was cooled in an ice bath. A solution of saturated sodium hydroxide solution

containing 3.8g of NaOH (95 mmol) was introduced very slowly into the mixture while maintaining the temperature at 10^0C. A white pasty mass appeared soon. After all of the alkaline solution was added, the pasty mass was disolved with 60 mL of cold water. The product is precipitated in 50mL of 14% hydrochloride acid solution. The solid is obtained by suction filtration and purified by recrystallization in etanol. The yield is 80%. Melting point 118 – 120^0C (118-120^0C[8]).

Synthesis of Pichromene 1

A mixture of 4-fluoro-β-nitrostyrene (50 mg, 0,3 mmol; 1 equiv), 3-ethoxysalicyl-aldehyde (50 mg; 0,3 mmol; 1 equiv), and L-pipecolic acid (8mg; 0,06 mmol; 20 mol%) was mostly dissolved by 1mL of dry toluene . The mixture was stirred at 80^0C for 24 hours (under Argon atmosphere). The reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The extracts were washed with brine, then dried with $MgSO_4$, and evaporated. Crude product was further purified using column chromatography (ethyl acetate/n-Hexane) to get the pure Pichromene 1 (1).

^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) = 1,36 (s, 1H, CH_3, J=7,1); 3,97-4,08 (m, 2H, OCH_2, J=7,1); 6,64 (s, 1H, H-1); 6,93-6,99 (m, 5H, H-3,4,5,7,8); 7,37 (d, 1H; J=5,2, H-6); 7,38 (d, 1H, J=5,2, H-9); 8,03 (s, 1H, H-2)

^{13}C NMR (CDCl3, 125 MHz): δ (ppm) = 164,2; 162,2; 148,0; 143,0; 141,3; 132,6; 129,5; 128,9; 128,8; 122,6; 122,2; 118,9; 118,7; 115,8; 115,6; 65,2 (OCH_2); 14,7 (CH_3).

Similarly have been synthesized other Pichromenes 2-9. The yields and some their physico-chemical characteristics are conducted in table 2 and 3.

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Tổng hợp tác nhân tiềm năng chống ung thư Pichromene, sử dụng xúc tác Bazơ hữu cơ

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Tóm tắt: Pichromene 1 là một chất có khả năng ức chế sự phát triển của tế bào ung thư, đang được nghiên cứu ứng dụng trong điều trị bệnh bạch cầu mãn tính. Trong công trình này nghiên cứu điều chế Pichrmene 1 và các dẫn xuất bằng phản ứng ngưng tụ giữa salicylaldehyde thế và các *β*-nitrostyrene thế trong những điều kiện (dung môi, thời gian phản ứng, nhiệt độ và xúc tác) khác nhau. Kết quả cho thấy, trong dung môi toluen, ở nhiệt độ 80^0C, với xúc tác là axit L-Pipecolic, hiệu suất Pichromene đạt đến 82% sau thời gian 24h.

Cấu trúc của các sản phẩm đã được xác định bằng các dự kiện phổ ^{1}H-NMR và ^{13}C- NMR.