

Synthesis and Screening of a Combinatorial Library of Naphthalene Substituted Chalcones: Inhibitors of Leukotriene B₄[†]

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Abstract—A combinatorial mini library of naphthalene substituted chalcones has been prepared by solution phase chemistry. Screening of these mixtures for leukotriene B₄ inhibitory activity using human whole blood assay (HWBL) afforded a lead compound, 1-(6-butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K₄A₃) with an IC₅₀ value of 18.5 μM. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Leukotrienes (LTs) are important mediators of smooth muscle constriction,¹ increased vascular permeability² and leukocyte chemotaxis.³ The enzyme 5-lipoxygenase (5-Lo) catalyzes the initial step in arachidonic acid cascade leading to LTA₄, the precursor to the family of LTs i.e. LTB₄, C₄, D₄ and E₄.^{3,4} Limiting the synthesis of LTs through inhibition of 5-Lo has provided a new therapeutic approach for treating a variety of inflammatory conditions including asthma, allergic rhinitis, rheumatoid arthritis, psoriasis and ulcerative colitis. In the past decade, a large number of organic compounds have been reported⁵ as 5-Lo inhibitors, but the majority of them exhibit either insufficient bioavailability or redox properties.⁶ Recently the chalcone derivatives^{7a–d} have also shown a promising 5-Lo inhibition with anti-inflammatory and anti-allergic activity. Based on the suggestion⁸ by Summer et al. on precise fitting of naphthalene skeleton in hypothetical arachidonic acid conformation, we report a combinatorial synthesis and biological evaluation of substituted naphthalene based chalcones.

Chemistry and Library Synthesis

The required ketones (K₂–K₄) were synthesized employing Friedel–Crafts acylation of corresponding 6-alkoxynaphthalenes according to the literature procedure.⁹ The library synthesis is based on simple and rapid Claisen Schmidt condensation reaction¹⁰ of

2-naphthyl methyl ketones (K₁ to K₄) with substituted benzaldehydes (A₁ to A₅) to furnish combinatorial mixtures (K_nA_n) free of byproducts (Scheme 1). Twenty compounds were synthesized in two sets as nine combinatorial mixtures (Table 1). In the first set each pure ketone (K₁ to K₄) was reacted with stoichiometric amount of equimolar mixture of aldehydes (A_{1–5}) and in another set each pure aldehyde (A₁ to A₅) was reacted with a stoichiometric amount of equimolar mixture of ketones (K₁–K₄). Although the reactivities of aldehydes (A₁–A₅) and ketones (K₁–K₄) are different, the reaction conditions employed for the synthesis of a combinatorial library were adequate for quantitative conversion (tlc, 48 h) of a mixture of ketones and aldehydes to obtain a diverse menu of chalcones for biological screening. The HPLC analysis revealed that the concentration of anticipated components in combinatorial mixtures are almost in the same proportion.

Biological Evaluation

The combinatorial mixtures were evaluated for their LTB₄ inhibitory activity by human whole blood assay¹¹ (HWBL). Inhibition for each combinatorial mixture at different concentrations was calculated and compared with the results of standard LTB₄ inhibitor Zileuton. IC₅₀ value was determined by regression analysis for the compound represented in hit mixture.

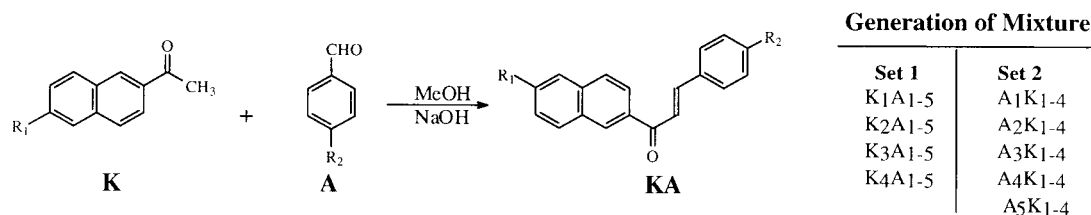
Library Screening

The library mixtures were screened using the method described in biological evaluation and results are depicted in Table 2. Two combinatorial mixtures (K₄A_{1–5}

Key words: Naphthalene chalcones; antiinflammatories; enzyme inhibitors; leukotrienes.

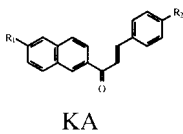
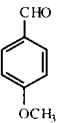
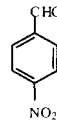
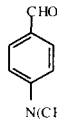
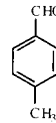
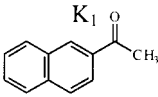
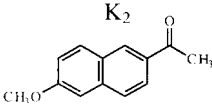
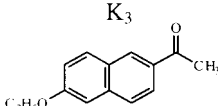
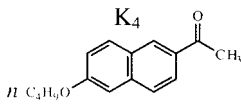
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Scheme 1.

Table 1. Combinatorial synthesis of naphthalene based chalcones

 KA	 A₁	 A₂	 A₃	 A₄	 A₅
 K₁	K ₁ A ₁	K ₁ A ₂	K ₁ A ₃	K ₁ A ₄	K ₁ A ₅
 K₂	K ₂ A ₁	K ₂ A ₂	K ₂ A ₃	K ₂ A ₄	K ₂ A ₅
 K₃	K ₃ A ₁	K ₃ A ₂	K ₃ A ₃	K ₃ A ₄	K ₃ A ₅
 K₄	K ₄ A ₁	K ₄ A ₂	K ₄ A ₃	K ₄ A ₄	K ₄ A ₅

and A₃K₁₋₄) each from one set, showed the highest LTB₄ inhibitory activity (31% and 37% at 30 μM concentration respectively), whereas the other mixtures gave an insignificant response as compared to the standard inhibitor, Zileuton. These mixtures showed either low or no inhibition with rather normal biological variation that is present in this assay. Considerable LTB₄ inhibitory activity of the combinatorial mixtures K₄A₁₋₅ and A₃K₁₋₄ indicated K₄A₃ as expected lead compound. The lead compound, 1-(6-butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (**K₄A₃**) was synthesized by condensing 6-butoxy-2-acetonaphthone with *p*-nitrobenzaldehyde, which on evaluation for LTB₄ inhibitory assay showed 62% inhibition at 30 μM concentration with an IC₅₀ of 18.5 μM. It appears that further manipulation of substituents, position of substituents and derivatisation of functional group may provide highly potent LTB₄ inhibitors and further studies are in progress.

In summary, we have synthesized a mini library of substituted naphthalene chalcones by solution phase

combinatorial chemistry and the biological evaluation results have provided a lead compound (K₄A₃) for further exploration to obtain a potent LTB₄ inhibitor.

Experimental

Aromatic aldehydes used for synthesis were procured from Aldrich Chemical Co. HPLC analysis was performed using C-18 reverse phase column in acetonitrile: water (7:3) mixture and measuring the absorbance at 254 nm.

Method for library preparation

0.5 M Stock solutions of individual reactant (K and A) were prepared in MeOH (20 mL). Solutions (10 mL) of all components from same reactant i.e. ketones (K₁–K₄) and aldehydes (A₁–A₅) were mixed separately to obtain K₁₋₄ & A₁₋₅. In case of ketones the solution of mixed components K₁₋₄ was diluted to 50 mL with MeOH to get 0.1 M of each reactant in solution. Solution (10 mL,

Table 2. Results of LTB₄ inhibitory activity

Combinatorial mixture	% Inhibition of LTB ₄ formation in human whole blood assay			
	Concentrations			
	1 μ M	3 μ M	10 μ M	30 μ M
K ₁ A _{1–5}	–4	–1	2	30
K ₂ A _{1–5}	25	12	15	20
K ₃ A _{1–5}	13	8	13	26
K₄A_{1–5}	–	10	24	31
A ₁ K _{1–4}	–6	16	26	19
A ₂ K _{1–4}	–14	–5	2	8
A₃K_{1–4}	20	27	25	37
A ₄ K _{1–4}	2	12	4	–2
A ₅ K _{1–4}	–1	–5	14	–
K₄A₃	23	25	36	62
Zileuton ^a	48	69	93	–

Lead compound and hit combinatorial mixtures are shown by bold face type.

^a = Standard Inhibitor, (IC₅₀ 0.93 μ M).

0.5 M) of individual reactant (K or A) and solution of mixed components of other reactants (A_{1–5} or K_{1–4}, 0.1 M, 10 mL) were mixed and aq. NaOH was added (0.5 M, 1 mL). The reaction mixtures were stirred at rt for 48 h, then concentrated to dryness in vacuo, neutralised with 1 N HCL and extracted with CHCl₃ (2 $\times$ 40 mL). The combined organic layers were washed with water, brine and dried over anhydrous Na₂SO₄, concentration in vacuo furnished gummy or solid products, in quantitative yield.

HPLC Analysis

The combinatorial mixtures K₂A_{1–5} and A₃K_{1–4} were analyzed by HPLC. The HPLC of combinatorial library mixtures was compared with the authentic mixtures prepared by mixing equimolar amounts of the compounds synthesized individually (K₂A₁ to K₂A₅ and A₃K₁ to A₃K₄). They showed identical HPLC profile.

1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K₄A₃). To a stirred solution of 6-butoxy-2-acetonaphthone (2.42 gm, 10 mmol) and *p*-nitrobenzaldehyde (1.51 gm, 10 mmol) in MeOH (10 mL) was added aq. NaOH (10 mmol, 2 mL). Reaction mixture was stirred at rt for 24 h. It was concentrated to dryness in vacuo, neutralized with 1 N HCL and extracted with CHCl₃ (2 $\times$ 25 mL). The combined organic layer was washed with water, brine and dried over Na₂SO₄ and concentration of organic layer in vacuo followed by the silica gel column chromatographic purification of the residue furnished pure **K₄A₃** in quantitative yield. ¹H NMR (CDCl₃, 200 MHz) δ 0.85 (t, *J* = 8 Hz, 3H), 1.20–1.50 (m, 2H), 1.50–1.80 (m, 2H), 4.15 (t, *J* = 7 Hz, 2H), 7.20–7.55 (m, 5H), 7.65 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 1H), 7.84 (d, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H), 8.23 (d, *J* = 8 Hz, 2H); MS (m/e) 375, 318, 291, 270, 255, 244, 227, 215, 197, 183, 170, 155, 142, 126, 115, 102, 89, 83, 71, 63, 57; IR $\nu_{\max}$ 1655, 1600 cm^{–1}. Anal. calcd. for C₂₃H₂₁NO₄: C, 73.60; H, 5.60; N, 3.73. Found: C, 73.48; H, 5.73; N, 3.57.

Compounds K₂A₁ to K₂A₅, A₃K₁ and A₃K₃ were synthesized by analogous method.

1-(6-Methoxy-2-naphthyl)-3-(phenyl)-prop-2-en-1-one (K₂A₁). Mp 124 °C, ¹H NMR (CDCl₃, 200 MHz) δ 3.95 (s, 3H), 7.05–7.20 (d, *J* = 14 Hz, 1H), 7.30–7.60 (m, 9H), 7.65–7.75 (d, *J* = 7 Hz, 1H), 7.80–7.90 (d, *J* = 7 Hz, 1H), 7.90–8.05 (d, *J* = 7 Hz, 1H); MS (m/e) 288, 271, 260, 245, 229, 211, 185, 170, 142, 127, 114, 103, 77, 63; Anal. calcd. for C₂₀H₁₆O₂: C, 88.33; H, 5.60. Found: C, 83.24; H, 5.72.

1-(6-Methoxy-2-naphthyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (K₂A₂). Mp 130 °C, ¹H NMR (CDCl₃, 200 MHz) δ 3.85 (s, 3H), 3.95 (s, 3H), 6.80–6.95 (d, *J* = 7 Hz, 1H), 6.95–7.10 (d, *J* = 14 Hz, 1H), 7.15–7.55 (m, 6H), 7.60–7.80 (m, 2H), 7.80–7.90 (d, *J* = 7 Hz, 1H), 7.90–8.00 (d, *J* = 7 Hz, 1H); MS (m/e) 318, 301, 290, 275, 259, 247, 211, 185, 170, 142, 127, 114, 101, 89, 77, 63; Anal. calcd. for C₂₁H₁₈O₃: C, 79.23; H, 5.70. Found: C, 79.34; H, 5.77.

1-(6-Methoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K₂A₃). Mp 200 °C, ¹H NMR (CDCl₃, 200 MHz) δ 3.95 (s, 3H), 7.20–7.30 (d, *J* = 14 Hz, 1H), 7.30–7.60 (m, 5H), 7.60–7.90 (m, 4H), 7.90–8.05 (d, *J* = 7 Hz, 1H), 8.15–8.30 (d, *J* = 7 Hz, 1H); MS (m/e) 333, 316, 290, 274, 259, 243, 228, 215, 197, 170, 157, 142, 127, 114, 102, 76, 63; Anal. calcd. for C₂₀H₁₅O₄: C, 72.06; H, 4.54; N, 4.20. Found: C, 72.00; H, 5.68; N, 4.31.

1-(6-Methoxy-2-naphthyl)-3-(4-N,N-dimethylanilino)-prop-2-en-1-one (K₂A₄). Mp 174 °C, ¹H NMR (CDCl₃, 200 MHz) δ 3.05 (s, 6H), 4.00 (s, 3H), 6.65–6.80 (d, *J* = 7 Hz, 2H), 7.10–7.30 (m, 2H), 7.45–7.70 (m, 3H), 7.75–8.00 (m, 3H), 8.05–8.20 (d, *J* = 7 Hz, 1H), 8.5 (s, 1H); Anal. calcd. for C₂₂H₂₁O₂: C, 79.73; H, 6.39; N, 4.23. Found: C, 79.45; H, 6.68; N, 4.31.

1-(6-Methoxy-2-naphthyl)-3-(4-methylphenyl)-prop-2-en-1-one (K₂A₅). Mp 165 °C, ¹H NMR (CDCl₃, 200 MHz) δ 2.40 (s, 3H), 3.95 (s, 3H), 7.15–7.35 (m, 4H), 7.50–7.65 (d, *J* = 7 Hz, 2H), 7.65–7.75 (s, 1H), 7.75–7.95 (m, 3H), 8.05–8.15 (d, *J* = 7 Hz, 1H), 8.5 (s, 1H); MS (m/e) 302, 287, 259, 243, 211, 197, 158, 127, 105, 91, 77, 65; Anal. calcd. for C₂₁H₁₈O₂: C, 83.41; H, 6.00. Found: C, 83.24; H, 6.37.

1-(2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K₁A₃). Mp 139 °C, ¹H NMR (CDCl₃, 300 MHz) δ 6.65–6.75 (d, *J* = 7 Hz, 2H), 7.30–7.75 (m, 6H), 7.75–8.00 (m, 3H), 8.05–8.15 (d, *J* = 9 Hz, 1H), 8.15–8.25 (d, *J* = 9 Hz, 1H); Anal. calcd. for C₁₉H₁₃O₃: C, 75.24; H, 4.32; N, 4.62. Found: C, 75.45; H, 4.68; N, 4.56.

1-(6-Ethoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K₃A₃). Mp 156 °C, ¹H NMR (CDCl₃, 200 MHz) δ 1.30–1.45 (t, *J* = 6 Hz, 3H), 4.15–4.35 (q, *J* = 6 Hz, 2H), 7.15–7.35 (d, *J* = 14 Hz, 1H), 7.35–7.60 (m, 5H), 7.60–7.90 (m, 4H), 7.90–8.05 (d, *J* = 7 Hz, 1H), 8.15–8.30 (d, *J* = 7 Hz, 1H); MS (m/e) 347, 290, 272, 255, 226, 197, 171, 143, 115, 102, 76, 63; Anal. calcd. for C₂₁H₁₇NO₄: C, 72.61; H, 4.93; N, 4.03. Found: C, 72.85; H, 5.08; N, 3.86.

5-Lo human whole blood assay.¹¹ Human blood was collected into heparinised blood collection tubes and aliquoted in 1 mL portion into 1.5 mL microfuge tubes. 5 μ L of test compound in DMSO was added to the blood sample and incubated for 15 min. at 37°C. Calcium ionophore A23187 (in DMSO, 50 μ M final concentration) and the sample were incubated for 30 min. at 37°C. The samples were centrifuged (1100 X g, 10 min. at 4°C) and supernatant was assayed for LTB₄ using an EIA kit (Cayman Chemical). All results are mean of duplicates and in most cases triplicate determination.

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