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Synthesis and Screening of a Combinatorial Library of Naphthalene Substituted Chalcones: Inhibitors of Leukotriene B_4†

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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 B_4 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 (K_4A_3) with an IC_{50} value of 18.5 μM.

Introduction

Leukotrienes (LTs) are important mediators of smooth muscle constriction,[1] increased vascular permeability[2] and leukocyte chemotaxis.[3] The enzyme 5-lipoxygenase (5-Lo) catalyzes the initial step in arachidonic acid cascade leading to LTA_4, the precursor to the family of LTs i.e.LTB_4, C_4, D_4 and E_4.[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 rhenitis, rheumatoid arthritis, psoriasis and ulcerative colitis. In the past decade, a large number of organic compounds have been reported[5] 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 suggestion[8] 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 (K_2–K_4) 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 Schimidt condensation reaction[10] of 2-naphthyl methyl ketones (K_1 to K_4) with substituted benzaldehydes (A_1 to A_5) 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 (K_1 to K_4) was reacted with stoichiometric amount of equimolar mixture of aldehydes (A_{1-5}) and in another set each pure aldehyde (A_1 to A_5) was reacted with a stoichiometric amount of equimolar mixture of ketones (K_1–K_4). Although the reactivities of aldehydes (A_1–A_5) and ketones (K_1–K_4) 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 LTB_4 inhibitory activity by human whole blood assay[11] (HWBL). Inhibition for each combinatorial mixture at different concentrations was calculated and compared with the results of standard LTB_4 inhibitor Zileuton. IC_{50} 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 (K_4A_{1-5}

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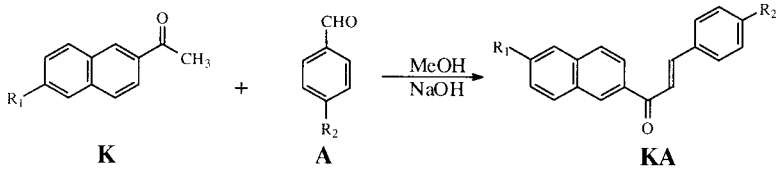

Generation of Mixture

Set 1	Set 2
$K_1A_{1\text{-}5}$	$A_1K_{1\text{-}4}$
$K_2A_{1\text{-}5}$	$A_2K_{1\text{-}4}$
$K_3A_{1\text{-}5}$	$A_3K_{1\text{-}4}$
$K_4A_{1\text{-}5}$	$A_4K_{1\text{-}4}$
	$A_5K_{1\text{-}4}$

Scheme 1.

Table 1. Combinatorial synthesis of naphthalene based chalcones

KA	A_1	A_2	A_3	A_4	A_5
K_1	K_1A_1	K_1A_2	K_1A_3	K_1A_4	K_1A_5
K_2	K_2A_1	K_2A_2	K_2A_3	K_2A_4	K_2A_5
K_3	K_3A_1	K_3A_2	K_3A_3	K_3A_4	K_3A_5
K_4	K_4A_1	K_4A_2	K_4A_3	K_4A_4	K_4A_5

and $A_3K_{1\text{–}4}$) each from one set, showed the highest LTB_4 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 LTB_4 inhibitory activity of the combinatorial mixtures $K_4A_{1\text{–}5}$ and $A_3K_{1\text{–}4}$ indicated K_4A_3 as expected lead compound. The lead compound, 1-(6-butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (**K_4A_3**) was synthesized by condensing 6-butoxy-2-acetonaphthone with *p*-nitrobenzaldehyde, which on evaluation for LTB_4 inhibitory assay showed 62% inhibition at 30 μM concentration with an IC_{50} of 18.5 μM. It appears that further manipulation of substituents, position of substituents and derivatisation of functional group may provide highly potent LTB_4 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 (K_4A_3) for further exploration to obtain a potent LTB_4 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 (K_1–K_4) and aldehydes (A_1–A_5) were mixed separately to obtain $K_{1\text{–}4}$ & $A_{1\text{–}5}$. In case of ketones the solution of mixed components $K_{1\text{–}4}$ was diluted to 50 mL with MeOH to get 0.1 M of each reactant in solution. Solution (10 mL,

Table 2. Results of LTB_4 inhibitory activity

Combatorial mixture	% Inhibition of LTB_4 formation in human whole blood assay			
	Concentrations			
	1 μM	3 μM	10 μM	30 μM
K_1A_{1-5}	−4	−1	2	30
K_2A_{1-5}	25	12	15	20
K_3A_{1-5}	13	8	13	26
K_4A_{1-5}	–	**10**	**24**	**31**
A_1K_{1-4}	−6	16	26	19
A_2K_{1-4}	−14	−5	2	8
A_3K_{1-4}	**20**	**27**	**25**	**37**
A_4K_{1-4}	2	12	4	−2
A_5K_{1-4}	−1	−5	14	–
K_4A_3	**23**	**25**	**36**	**62**
Zileuton[a]	48	69	93	–

Lead compound and hit combinatorial mixtures are shown by bold face type.
[a] = Standard Inhibitor, (IC_{50} 0.93 μM).

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 $CHCl_3$ (2×40 mL). The combined organic layers were washed with water, brine and dried over anhydrous Na_2SO_4, concentration in vacuo furnished gummy or solid products, in quantitative yield.

HPLC Analysis

The combinatorial mixtures K_2A_{1-5} and A_3K_{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 (K_2A_1 to K_2A_5 and A_3K_1 to A_3K_4). They showed identical HPLC profile.

1-(6-Butoxy-2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K_4A_3). 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 $CHCl_3$ (2×25 mL). The combined organic layer was washed with water, brine and dried over Na_2SO_4 and concentration of organic layer in vacuo followed by the silica gel column chromatographic purification of the residue furnished pure **K_4A_3** in quantitative yield. ^1H NMR ($CDCl_3$, 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 ν_{max} 1655, 1600 cm^{-1}. Anal. calcd. for $C_{23}H_{21}NO_4$: C, 73.60; H, 5.60; N, 3.73. Found: C, 73.48; H, 5.73; N, 3.57.

Compounds K_2A_1 to K_2A_5, A_3K_1 and A_3K_3 were synthesized by analogus method.

1-(6-Methoxy-2-naphthyl)-3-(phenyl)-prop-2-en-1-one (K_2A_1). Mp 124 °C, ^1H NMR ($CDCl_3$, 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 $C_{20}H_{16}O_2$: 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 (K_2A_2). Mp 130 °C, ^1H NMR ($CDCl_3$, 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 $C_{21}H_{18}O_3$: 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 (K_2A_3). Mp 200 °C, ^1H NMR ($CDCl_3$, 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 $C_{20}H_{15}O_4$: 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 (K_2A_4). Mp 174 °C, ^1H NMR ($CDCl_3$, 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 $C_{22}H_{21}O_2$: 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 (K_2A_5). Mp 165 °C, ^1H NMR ($CDCl_3$, 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 $C_{21}H_{18}O_2$: C, 83.41; H, 6.00. Found: C, 83.24; H, 6.37.

1-(2-naphthyl)-3-(4-nitrophenyl)-prop-2-en-1-one (K_1A_3). Mp 139 °C, ^1H NMR ($CDCl_3$, 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 $C_{19}H_{13}O_3$: 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 (K_3A_3). Mp 156 °C, ^1H NMR ($CDCl_3$, 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 $C_{21}H_{17}NO_4$: 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 μL 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 μM 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 LTB_4 using an EIA kit (Cayman Chemical). All results are mean of duplicates and in most cases triplicate determination.

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