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Synthesis and biochemical evaluation of benzyl propargyl ethers as inhibitors of 5-lipoxygenase

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A series of benzyl propargyl ethers were synthesized and tested as inhibitors of 5-lipoxygenase, the key enzyme involved in leukotriene biosynthesis. Among these, optimum activity was displayed by 1-(2-heptynyloxymethyl) benzene **12** (IC_{50} 1.2 μM). Addition of carboxyl group at the end of the alkyl side chain attached to the acetylenic group abolished the inhibition. Selective reduction of the acetylenic group to *cis* or *trans* double bond reduced the inhibitory potential, the *cis* isomer **24** showing more than 20-fold higher inhibition than the *trans* isomer **25**. Introduction of sulphur in place of oxygen in the alkyl side chain attached to the (carboxyalkyl) benzyl group also reduced the inhibition. The IC_{50} value of **12**, towards rabbit reticulocyte 15-LOX is > 50 fold higher than that of 5-LOX. These results indicate that compound **12** is a specific inhibitor of 5-LOX.

Introduction

Lipoxygenases (LOXs - EC 1.13.11.33) comprise a family of non-heme iron containing dioxygenases that catalyze the incorporation of molecular oxygen into polyunsaturated fatty acids such as arachidonic acid (AA) and linoleic acid (LA) to generate the corresponding hydroperoxy fatty acids. Based on the position of incorporation of molecular oxygen on AA, LOXs are classified as 5-, 8-, 11-, 12- and 15-lipoxygenases. Leukotrienes, 5-LOX metabolites of arachidonic acid, have been implicated as mediators of allergy, asthma[1] and a number of other inflammatory disorders like rheumatoid arthritis, inflammatory bowel disease, psoriasis and glomerulonephritis[2-4]. 15-LOX, localized in reticulocytes and airway epithelial cells[5] produces 15(S)-HPETE, which is converted to 15(S)-HETE. 15(S)-HETE is suspected to be involved in counteracting the inflammatory reaction of the leukotrienes[6]. The unique characteristic feature of the 15-LOX is its ability to oxidize LDL, which might play a pivotal role in the primary stage of atheroma formation[7]. Similarly 12-lipoxygenase expressions have been well documented in many types of solid tumour cells, including those of prostate, colon and epidermoid carcinoma[8,9]. Also, studies have shown that 12(S)-HETE is a critical intracellular signaling molecule, stimulating protein kinase C and eliciting the biological actions of many growth factors and cytokines[10,11]. Thus lipoxygenase (5-, 12- and 15-) - derived products have been involved in the pathogenesis of a variety of human diseases. As a result, several attempts have been made in the last decade to identify and develop specific inhibitors of 5-, 12- and 15-LOXs that could form potential therapeutic agents. These efforts have resulted in the release of Zileuton (5-LOX inhibitor) and Montelukast (leukotriene receptor antagonist) into the market for the treatment of asthma[1]. Recent studies have indicated that LOX inhibitors may be superior to leukotriene-receptor antagonists, as they block the action of the full spectrum of 5-LOX products whereas leukotriene receptor antagonists would produce narrower effects.

Lipoxygenase inhibitors reported in the literature have been classified as arachidonic acid related inhibitors, catechols and related substances, iron chelators, substrate analogues, naphthols, pyrazoline derivatives of 5-LOX activating protein (FLAP) inhibitors, hydroxamic acid and N-hydroxy urea type inhibitors, antioxidant based inhibitors and miscellaneous type[12,13]. The acetylenic fatty acid

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Abbreviations used: LOXs, lipoxygenases; HPETE, hydroperoxyeicosatetraenoic acid, HETE, hydroxyeicosatetraenoic acid; AA-Arachidonic acid; LA, linoleic acid; LDL, low density lipoprotein; DMSO, dimethyl sulphoxide; THF, tetrahydrofuran; TLC, thin layer chromatography

analogues interfere the biogenesis by getting oxidized and behave as suicidal substrates. Earlier, Gorins *et al.*[14] have demonstrated (carboxyalkyl) benzyl propargyl ethers as selective inhibitors of leukocyte type 12-lipoxygenases. In the present study, a series of acetylenic substrate analogues that might serve as mechanism-based inhibitors were synthesized and tested for inhibition of 5-lipoxygenase.

Materials and Methods

Linoleic acid and arachidonic acid were obtained from Sigma Chemical Company, USA and other chemicals used were obtained from indigenous companies.

Preparation of Enzyme

5-Lipoxygenase

The 5-LOX enzyme, which is primarily expressed in mammalian cells of myeloid origin, is also found in potato tubers[15]. Potato lipoxygenase is functionally very similar to the mammalian 5-LOX to generate 5-hydroperoxyeicosatetraenoic acid (5-HPETE) (Fig. 1) and is generally employed for inhibitor screening[16,17]. Therefore, studies on 5-LOX activity were carried out on potato 5-lipoxygenase enzyme. The enzyme was extracted from potato tubers as per the methods described earlier[18].

15-Lipoxygenase

The 15-LOX was extracted and purified from rabbit reticulocytes as per the methods described by Kuhn *et al.*[19].

Lipoxygenase assay

LOX was measured polarographically using Gilson Oxygraph. The reaction mixture in a final volume of 1.6 ml contained 0.15 *M* potassium phosphate buffer, *p*H 6.3, enzyme protein and 75 μ*M* arachidonic acid or 90 μ*M* linoleic acid. The reaction was initiated by the addition of the substrate and the reaction velocity calculated based on the slope obtained on oxygraph. LOX activity was expressed as μ moles O_2 consumed/min/mg protein.

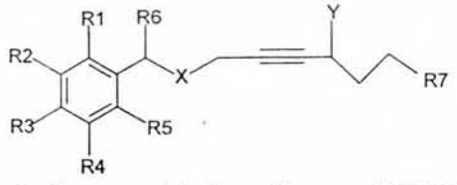


Fig. 1—Structure of the Parent Compound 5-HPETE

Inhibitor assays

The effect of different concentrations of compounds on LOX activity was examined under the same experimental conditions described above. LOX activity measured in the presence and absence of different compounds was taken for the determination of IC_{50} (the concentration of the compound that inhibits 50% of LOX activity) values.

General procedure for the preparation of 1-(2-propynyloxymethyl) benzene and its aryl substituted derivatives

KOH (4.2 g, 64.3 mmol) was crushed in a mortar and added to 250 ml 2-neck round bottom flask followed by the addition of DMSO (30 ml). Benzyl alcohol (57.9 mmol) was added to the crushed KOH and stirred for 30 min at room temperature.

Propargyl bromide (6.9 g, 5.79 mmol) was added slowly to the above reaction mixture. After 3 hr reaction mixture was diluted with water and the product was extracted with ethyl acetate, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic purification furnished the pure product in high yields. 1N-benzyl, 1N-methyl, 2-propyn-1-amine and 1-(2-propynyloxysulfanylmethyl) benzene were also prepared by the same method by condensation of N-methyl benzylamine or benzylthiol with propargyl bromide. 2,3-Dimethyl phenol, butane-, butene- or butynediol were converted to their corresponding dipropargyl ethers by the same method using 2.2 equivalents of KOH and propargyl bromide.

Disubstituted compounds **1-7** were prepared from the corresponding propargyl ethers prepared as above. They were dissolved in dry THF (10 ml), cooled to –78°C and *n*-BuLi (2.1 equivalents of 15% solution in *n*-hexane) was added, stirred for 30 min. Freshly distilled *n*-butyraldehyde (2.2 equiv) or *n*-butylbromide (2.2 equiv) was added to the reaction mixture. The reaction temperature was maintained at –78°C for 3 hr and the progress of the reaction was monitored by TLC (silica gel; combination of petroleum ether: ethyl acetate in a ratio of 8:2 or 9.8:0.2 respectively as solvent system) and after completion of reaction, the mixture was slowly warmed to room temperature. Reaction mixture was quenched with saturated ammonium chloride solution and the product was extracted with ether, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic

purification using silica gel column and petroleum ether : ethyl acetate as solvent system for elution furnished the pure products.

Compound No. 1

Thick yellow liquid, 52.5%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, j = 6Hz, 6H), 1.35-1.65 (m, 8H), 2.15-2.25 (m, 4H), 3.75 (s, 3H), 4.05-4.15 (m, 4H), 4.60-4.70 (m, 4H), 6.75(d, j = 8Hz, 1H), 6.95 (d, j = 8Hz, 1H), 7.25 (dd, J_1= 8Hz, J_2 = 7Hz, 1H). MS (m/z): 356 (M^{+2},0.5), 299 (0.5), 244 (5), 242 (3), 227 (2), 188 (62), 149 (92), 91(100).

Compound No. 2

Thick yellow liquid, 24% (obtained as byproduct in the preparation of **1**), ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, j = 7Hz, 3H), 1.45-1.60 (m, 4H), 2.20 - 2.30 (m, 2H), 3.85 (s, 3H), 4.15-4.25 (m, 4H), 4.70-4.80 (m, 4H), 6.85(d, j = 8Hz, 1H), 7.05 (d, j = 8Hz, 1H), 7.25 (dd, J_1=7Hz, J_2 = 8Hz, 1H). MS (m/z): 300 (M^++2,4), 229 (7), 215 (4), 201 (5), 188 (22), 173 (12), 165 (118), 149 (58), 134 (32), 105 (28), 91 (100), 77 (75).

Compound No.3

Thick yellow liquid, ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, j = 8Hz, 3H), 1.35-1.75 (m, 4H), 2.40 (m, 1H), 3.75 (s, 3H), 4.05-4.15 (m, 4H), 4.30-4.40 (m, 1H), 4.60-4.80 (m, 4H), 6.80 (d, j = 8Hz, 1H), 6.95(d, j = 8Hz, 1H), 7.15-7.25 (m, 1H). MS (m\z): 316 (M^++2,0.5), 279(0.5), 260 (2), 227 (4), 213 (4), 199 (4), 188 (22), 173 (14), 159 (15), 149 (100), 134 (22), 121(22), 105(32), 91 (100).

Compound No. 4

Thick liquid, yield 46.8%. ^{1}H NMR (90MHz,$CDCl_3$): δ 0.90 (t, J = 7Hz, 6H), 1.30-1.80 (m, 8H), 3.65 (s, 3H), 3.75 (s, 4H), 4.15 (s 4H), 4.70 (d, J = 4Hz, 4H), 6.80 (d, j = 7Hz, 1H), 6.90 (d, j = 7Hz, 1H), 7.15 (d, J = 7Hz, 1H). MS (m/z): 387 (M^++1,0.5), 316(0.5), 285(1), 260(8), 243(5), 227(5), 213(10), 199(9), 189 (10), 171(12), 165(30), 149(100), 134(31), 121(30), 91(75).

Compound No. 5

Thick liquid, yield 88%, IR (neat): 1220, 2900, 3120, 3430 cm^{-1}. ^{1}H NMR (200MHz,$CDCl_3$): δ 0.85 (t, J=8Hz, 6H), 1.25-1.50 (m, 4H), 1.55-1.65 (m, 8H), 2.25 (s, 2 OH), 3.40-3.50 (m, 4H), 4.05 (s, 4H), 4.25-4.35 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): 13.76, 18.48, 26.11, 39.78, 56.26, 51.92, 69.67, 80.50, and 87.70. MS (m/z): 308 (M^+, 0.5), 267(2), 249(1), 183(10), 139(21), 111(8), 93(25), 71(100), 55(42).

Compound No. 6

Thick liquid, yield 87%, IR (neat): 740, 1060, 1680, 2960, 3380 cm^{-1}. ^{1}H NMR (200MHz, $CDCl_3$): δ 0.85 (t, J=7 Hz, 6H), 1.35-1.50 (m, 4H), 1.55-1.65 (m, 4H), 4.05-4.15 (m, 8H), 4.30 (t, J=6Hz, 2H), 5.15-5.20 (m, 2H). MS (m/z): 306 (M^+, 0.5), 292 (2), 263(3), 252(4), 245(6), 227(12), 193(30), 180(45), 165(45).

Compound No. 7

Thick liquid, yield 87%, IR (neat): 730, 1110, 2970, 2960, 3400 cm^{-1}. ^{1}H NMR (200MHz,$CDCl_3$): δ 0.90 (t, J=7 Hz, 6H), 1.30-1.80 (m, 8H), 2.75 (s, 2 OH), 4.20 (s, 8H), 4.30-4.40 (m, 2H). MS (m/z): 306 (M^+ + 2, 0.5), 280 (0.5), 256(3), 245(3), 228(2), 215(3), 203(3), 187(4), 165(6), 149(18), 71(65), 55(100).

*General procedure for the preparation of 1-benzyloxy-2-heptyn-4-ol (**8**) or 1-(2-heptynyloxy-methyl) benzene (**12**) and their analogues*

Benzyl propargyl ether ((Scheme 1, R to R4 = H, 6.85 mmol, prepared as above) was dissolved in dry THF (10 ml), cooled to –78°C and *n*-BuLi (3.2 ml of 15% solution in *n*-hexane, 7.5 mmol) was added, stirred for 30 min. Freshly distilled *n*-butyraldehyde (540 mg, 7.5 mmol) or *n*-butyl bromide (1.02 g, 7.2 mmol) was added to the reaction mixture. The reaction temperature was maintained at –78°C for 3 hr and monitored by TLC (silica gel; combination of petroleum ether : ethyl acetate in a ratio of 8:2 or 9.8:0.2 respectively as solvent system) and slowly warmed to room temperature. Reaction mixture was quenched with saturated ammonium chloride solution and the product was extracted with ether, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic purification furnished the pure product 1-benzyloxy-2-eptyn-4-ol **8** or 1-(2-heptynyloxymethyl) benzene **12** in yields as indicated below. Aza and thia derivatives **17** and **18** (Table 1) were also prepared by the same method.

Compound No. 8

Colorless liquid, yield 73%, IR ($CHCl_3$): 3600, 2980, 1455, 1065, 708 cm^{-1}. ^{1}H NMR (200 MH, $CDCl_3$): δ 1.00 (t, J=6Hz, 3H), 1.40-1.65 (m, 2H), 1.70-1.80 (m, 2H), 2.25 (s,OH), 4.25 (s, 2H), 4.35-4.45 (m, 1H), 4.65 (s, 2H), 7.25-7.45 (m, 5H). MS (m/z): 218 (M^+, 6), 203(6), 199(5), 189(12), 175(21), 175(21), 171(22), 157(11), 145(100), 117(32), 91(42).

Table 1—Monoether of type 8 and analogues

S. No.	Compd. no.	R1	R2	R3	R4	R5	R6	R7	X	Y	IC_{50} (μM)
1	8	H	H	H	H	H	H	CH_3	O	OH	**760**
2	9	H	H	CH_3	H	H	H	CH_3	O	OH	**45**
3	10	H	H	OMe	H	H	H	CH_3	O	OH	NI
4	11	H	H	Cl	H	H	H	CH_3	O	OH	NI
5	12	H	H	H	H	H	H	CH_3	O	H	**1.2**
6	13	H	H	OMe	H	H	H	CH_3	O	H	NI
7	14	H	H	Cl	H	H	H	CH_3	O	H	NI
8	15	H	OMe	OMe	OMe	H	H	CH_3	O	H	NI
9	16	H	H	Cl	H	H	Ph	CH_3	O	H	NI
10	17	H	H	H	H	H	H	CH_3	N-Me	H	NI
11	18	H	H	H	H	H	H	CH_3	S	H	NI
12	19	H	H	H	H	H	=O	CH_3	O	H	**64**
13	20	H	H	H	H	H	H	CO_2Me	O	H	NI
14	21	Me	H	H	H	H	H	CO_2Me	O	H	NI
15	22	Me	OMe	H	H	H	H	CO_2Me	O	H	NI
16	23	CH_2Br	OMe	H	H	H	H	CO_2Me	O	H	NI
17	24*	H	H	H	H	H	H	CH_3	O	H	**22**
18	25**	H	H	H	H	H	H	CH_3	O	OH	**540**

Reduction of Acetylenic bond to = * *cis* isomer of 12th compound;
** *trans* isomer of 12th compound
NI = No inhibition

Compound No. 9

Colorless liquid, yield 74%, ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.80 (t, J = 7Hz, 3H), 1.20-1.60 (m, 4H), 1.70-1.80 (s, OH), 2.20 (s. 3H), 4.00 (s, 2H), 4.45 (s, 2H), 6.9-7.1 (m, 4H). MS (m/z): 232 (M^+, 4), 217(2), 199(5), 185(6), 171(5), 159(52), 143(13), 131(35), 121(70), 105(100), 91(72), 79(72), 69(28).

Compound No. 10

Colorless liquid, yield 74%, ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.95 (t, J = 6Hz, 3H), 1.30-1.85 (m, 4H), 3.75 (s, 3H), 4.0 (bs. 2H), 4.45 (t, J=2H), 4.45 (t, J=2H, 1H), 4.50 (s, 2H), 6.85 (d, J=18Hz, 2H), 7.15 (d, J= 18Hz, 2H). MS (m/z): 248 (M^+, 5), 229(1), 215(2), 201(8), 187(3), 175(31), 160(8), 147(15), 135(55), 121(100), 109(48), 94(22), 77(47).

Compound No. 11

Pale yellow liquid, Yield 69%, IR ($CHCl_3$) 3610, 3600 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, J=6Hz, 3H), 1.30-1.50(m, 2H), 1.55-1.70 (m, 2H), 1.05 (s, OH), 4.10 (s, 2H), 4.30-4.35 (m, 1H), 4.45 (s, 2H), 7.15-7.30 (m, 4H). MS (m/z): 252 (M^+, 2), 236(1), 217(1), 205(4), 191(4), 179(18), 163(4), 151(4), 142(35), 125(71), 113(2), 107(59), 89(40), 77(100), 69(42).

Compound No. 12

Colorless liquid, yield 79%, IR (neat): 2957, 237, 1605, 1070, 605 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): 0.80 (t, J=9Hz, 3H), 1.25-1.50 (m, 4H), 2.10-2.20 (m, 2H), 4.1 (s, 2H), 4.55 (s, 2H), 7.20 - 7.30 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): 13.20, 18.00, 22.00, 30.00, 57.50, 71.20, 75.80, 86.50, 127.20, 128.10, 128.50, 137.00. MS (m/z): 202 ($M^{+\cdot}$ 0.5), 182(3), 159(3), 145(21), 105(29), 91(100), 79(38), 65(45).

Compound No. 13

Colorless liquid, yield 80%, ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.95 (t, J=6Hz, 3H), 1.40-1.75(m, 4H), 2.20-2.40 (m, 2H), 3.85 (s. 3H), 4.10-4.15 (m, 2H), 4.50 (s, 2H), 6.85 (d, J = 8Hz, 2H), 7.25 (d, J = 8Hz, 2H). MS (m/z): 233 (M^+ +1,4), 232 (M^+, 25), 231 (25), 217 (1), 201 (20), 189 (15), 175 (50), 136 (100), 121 (55), 109 (20), 77 (19).

Compound No. 14

Pale yellow liquid, yield 81%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 6Hz, 3H), 1.35-1.65(m, 4H,), 2.20-2.30 (m, 2H), 4.10 (s, 2H), 4.55 (s, 2H), 7.25-7.35 (m, 4H), MS (m/z): 238 (M^+, +1,5), 237(M, +7), 236(21), 235(36), 207(4), 201(18), 193(21), 181(32), 179(100), 165(4), 151(5), 139(28), 125(25), 91(5), 77(6).

Compound No. 15

Thick liquid, yield 79%, IR (neat): 2935,2233,1593

cm-1. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J=6Hz, 3H), 1.35-1.55 (m, 4H,), 2.25 (m, 2H), 3.80(s, 3H), 3.85 (s, 6H), 4.15(t, 2H), 4.50 (s, 2H), 6.55(s, 2H).^{13}C NMR (50Hz, $CDCl_3$) : 13.58, 18.49, 21.96, 30.75, 56.10, 57.80, 60.77, 71.54, 75.84, 87.37, 105.0, 133.47, 137.66, 153.31. MS (m/z): 293 (M^+, +1,5), 292(22), 277(2), 261(4), 235(6), 196(20), 184(20), 182(100), 167(30), 151(44).

Compound No. 16

Thick liquid, yield 78%, IR (neat):2933, 2233,1660cm-1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, J=6Hz, 3H), 1.35-1.60 (m, 4H,), 2.20-2.30 (m, 2H), 4.10-4.15 (m, 2H), 5.65 (s, 1H), 7.25-7.35 (m, 9H). MS (m/z): 312 (M^+, +2), 311(3), 269(8), 255(31), 235(100), 217(35), 201(78), 165(75), 139(35), 105(30).

Compound No. 17

Pale yellow liquid, yield 79%, IR (neat): 2931,2245,1610cm-1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 6Hz, 3H), 1.40 -1.65(m, 4H,), 2.20-2.30 (m, 2H), 2.25-2.30 (m, 2H), 2.35 (s, 3H), 2.60(s, 2H), 7.25-7.40 (m, 5H). MS (m/z): 213 (M^+, -2, 0.5), 176(4), 132(5), 199(2), 107(1), 95(92), 67(100), 53(85).

Compound No. 18

Pale yellow liquid, yield 59%, ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (t, J=7Hz, 3H), 1.35-1.55 (m, 4H,), 2.20-2.30 (m, 2H), 3.05-3.10 (m, 2H), 3.85 (s, 2H), 7.20-7.40 (m, 5H). MS (m/z): 219 (M^+ + 1,2), 218 (M^+, 22), 203(2), 185(3), 176(12), 161(15), 143(5), 129(11), 122(24), 91(100), 85(12), 65(32).

Compound No. 19

2-Heptyn-1-ol (1g, 8.9 mmol) was taken in dry THF (10 ml), cooled to 0°C and triethyl amine (0.95 g, 9.8 nmol) was slowly added to it. After 5 mins, benzoyl chloride (1.25 g, 8.9 nmol) was added drop wise to the reaction mixture and stirred for 2 hr at 0°C. It was slowly warmed to room temperature. Organic layer was washed with water followed by brine, dried over sodium sulphate and concentrated to give crude product. Column chromatographic purification furnished the pure 2-heptyl benzoate **19** as colorless oil (1.5 g, 84% yield). IR (Neat): 2880, 1695,1580,1240,695cm-1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, J = 10Hz, 3H), 1.35-1.55 (m, 4H,), 2.15-2.25 (m, 2H), 4.90 (s, 2H), 7.35-2.45 (m, 2H), 7.50-7.60 (m, 1H), 8.10-8.15 (m, 2H). MS (m/z): 216 (M^+, 1), 201 (0.5), 173(12), 159(2), 129(5), 105(100), 91(2), 77(28).

Compounds 20, 21, 22, and 23 were prepared as follows:

Sodium hydride (90 mg, 3.74 mmol) was washed twice with dry pet ether and dry THF (10 ml) was added under inert atmosphere. 7-Hydroxy-5-heptanoic acid methyl ester (583 g, 3.74 mmol) dissolved in THF (2 ml) was added at 0°C slowly to the reaction mixture and the reaction mixture was stirred at 0°C for 30 min. Then benzyl bromide (75 g 4.11 mmol) in dry THF (2 ml) was added slowly to the above reaction and the reaction mixture was stirred for 2 hr at the same temperature. The reaction temperature was maintained at –78°C for 3 hr and the progress of the reaction was monitored by TLC (silica gel; combination of petroleum ether: ethyl acetate in a ratio of 9:1 as solvent system) and after completion of reaction the mixture was slowly warmed to room temperature and sodium salt was quenched by saturated ammonium chloride solution. The product was extracted with ethyl acetate, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic purification furnished pure 7-benzyloxy 5-heptynoic acid methyl ester (**20**, 1.0 g, 64%).

Compounds methyl 7-(2-methylbenzyloxy)-5-heptynoate (21), methyl 7-(3- methoxy-2-methylbenzyloxy)-5-heptynoate (22) and methyl 7-(3-bromomethyl-3-methoxyl benzyloxy) 5-heptynoate (23) were obtained by the same method.

Compound No. 20

Colorless liquid, yield, 64%, IR (Neat): 3110, 2240, 1730, 1640, 1200 cm-1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.90 (m, 2H), 2.35 (m, 2H), 2.55 (t, J=7Hz, 2H), 3.70 (s, 3H), 4.15 (t, J=1Hz, 2H), 4.50 (s, 2H), 7.35 (m, 5 H). MS (m/z): 246 (M^+, 0.5) 185(12), 169(5), 154(21), 122(22), 105(45), 91(100).

Compound No. 21

Pale yellow liquid, yield, 62%, IR (Neat): 3150, 2320, 1640 cm-1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.55-1.65 (m, 4H), 1.85-1.95 (m, 4H), 2.35-2.55 (m, 4H), 2.35-2.55 (m, 8H), 3.70 (s. 6H), 4.15-4.25 (m, 4H), 4.70 (t, J=6Hz, 6H), 7.25-7.45 (m, 8H).

Compound No. 22

Colorless liquid, yield, 45%, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.70-1.85 (m, 4H), 2.20-2.50 (m, 14H), 3.65 (s, 6H), 3.75 (2s, 6H), 4.10(2s, 4H), 4.60 (2s, 4H), 4.60(2s, 4H), 6.50-6.70 (m, 4H), 7.05-7.40 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): 18.48, 19.27, 19.48, 24.02, 33.02, 51.72, 55.95, 56.14, 58.09, 58.23, 62.79, 63.15, 76.64, 77.27, 77.72, 77.90, 85.36, 85.69,

108.62, 110.20, 117.15, 123.02, 124.20, 126.18, 129.24, 132.99, 140.27, 157.73, 158.60, 173.76.

Compound No. 23

Colorless liquid, yield, 65%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65 (t, J = 9.0, 4H), 2.10-2.55 (m, 8H), 3.55 (s, 6H), 3.75 (s, 6H), 3.75 (s, 6H), 4.05 (s, 2H), 4.10(s, 2 H), 4.50(s, 2H), 4.55 (s, 2H), 5.10(s, 4H), 6.20-6.75(m, 4H), 6.90-7.30(m, 2H).

Preparation of Compound 24: (Scheme 3)

Step 1

Ethyl (di-o-tolylphosphono) acetate was prepared by the reported method[15].

Step 2

Ethyl-2- (Z)-heptenoate was prepared as follows:

Sodium hydride (332 mg, 50% dispersion, 6.91 mmol) was washed by dry pet ether under inert atmosphere and dry THF (20 ml) was added to the flask. Reaction mixture was cooled to –78°C and at the same temperature, ethyl (di-o-tolylphosphono) acetate, (2 g, 5.76 mmol) in dry THF (5 ml) was added drop-wise to the above cooled solution and the reaction mixture was stirred for 2 hr. The contents of the flask were slowly warmed to room temperature and quenched by saturated ammonium chloride solution. The product was extracted with ether, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic purification furnished the pure product ethyl 2-(Z)-heptenoate, as colorless oil, (863 mg in 96% yield). IR: 2958, 1722, 1643, 1180, 729 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 8 Hz, 3H), 1.35-1.40 (t, J = 6Hz, 3H), 1.40-1.50 (m, 4H), 2.55-2.75 (m, 2H), 4.15 (q, J = 6 Hz, 2H), 5.75 (dt, J = 12Hz, 1H), 6.20 (dt, J = 12 and 8Hz, 1H). MS (m/z): 156 (M^+, 15), 141(2), 127(22), 111(80), 99(62).

Step-3: 2-(Z)-Hepten-1-ol

Ethyl 2-(Z)-heptenoate (500 mg, 3.2 mmol, from step-2) was dissolved in toluene (10 ml) under Nitrogen atmosphere and cooled to –78°C and at the same temperature DIBAL-H (2.6 ml, 2.5 molar in toluene solution, 6.4 mmol) was slowly added. Reaction mixture stirred for 2 hr at the same temperature. Aluminium complex was quenched by slow addition of methanol (5 ml) at the same temperature. Reaction mixture was slowly warmed up to room temperature and product was extracted 7-8 times by hot methanol. Removal of the solvent furnished crude alcohol, which on column chromatographic purification on neutral alumina furnished pure 2-(Z)-hepten-1-ol (266 mg, 72% yield) as a colorless oil. IR: 3330, 2958,1655, 1465, 696 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$: δ 0.90 (t, J = 6Hz, 3H), 1.25-1.40 (m, 4H), 1.80 (s, OH), 2.05-2.15 (m, 2H), 4.15 (d, J = 5 Hz, 2H), 5.45-5.65 (m, 2H), MS (m/z): 114 (M^+, 15), 96(13), 85(27), 81(33), 57(100).

Step-4: 1- [(Z)-2-Heptenyloxymethyl] benzene (24)

Sodium hydride (231 mg, 50% dispersion, 4.8 mmol) was washed with dry pet ether and dry THF (10 ml) was added to it under inert atmosphere. Reaction mixture was cooled to 0°C and at the same temperature, *cis* alcohol (from step-3) (500 mg, 4.54 mmol), in dry THF (2 ml) was slowly added and stirred for 30 min. Benzyl bromide (0.9 g, 5.26 mmol) in dry THF (2 ml) was added and the reaction mixture was stirred for 2 hr at same temperature. After 2 hr product was extracted with ethyl acetate, washed with brine, dried over sodium sulfate and concentrated to give crude product. Column chromatographic purification (neutral alumina) furnished the pure product (796 mg, 89% yield) as colorless oil. IR: 2956, 1620, 1496, 1454, 1095, 607 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 3 Hz, 3H), 1.37 (m, 4H), 2.10 (m, 2H), 4.10 (d, J = 1 Hz, 2H), 4.55 (s, 2H), 5.65 (m, 2H), 7.37 (m, 5H). MS (m/z): 204 (M^+, 5), 175 (2), 160 (30), 147(21), 129(5), 113(65), 117(100). Microanalysis: expected for $C_{14}H_{20}O$, C = 82.3, H = 9.61, O = 8.09 Found: C = 82.71, H = 9.89, O=7.40.

Preparation of compound 25: (Scheme 4)

Compound **25** was prepared by the same sequence of reaction as above for **24** except that ethyl (diethoxyphophono) acetate was used in the step 1. *n*-BuLi was used as base instead of sodium hydride for the preparation of ethyl to (E)-heptenoate.

Ethyl 2(E)-heptenoate

Colorless oil. IR: 2958, 1722, 1643,823 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 8Hz, 3H), 1.35–1.40 (t, J = 7Hz, 3H), 1.40-1.50 (m. 4H), 2.20 (m, 2H), 4.10-4.20(q, J = 6Hz, 2H), 5.75 (dt, J =12 and 2Hz 1H), 6.95 (dt, J =16 Hz and 6Hz, 1H). MS (m/z): 1.56 (M^+, 15), 142(2), 127(22), 111(80), 99(62).

2(E)-Hepten-1-ol

Colorless oil. yield, 70%, IR: 3280, 2956, 1660, 960, 770 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 8Hz, 3H), 1.30-1.50 (m, 4H), 2.05 (m, 2H), 2.7 (s, OH), 4.05 (d, J = 5Hz, 2H), 5.60-5.70 (m, 2H). MS (m/z): 114(M^+, 5), 96(18), 85(7), 81(35), 57(100).

1-[(E)-Heptenyloxymethyl] benzene (25)

Colorless oil. yield, 85%, IR (Neat) 2854, 1622, 1454, 970, 696 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 6Hz, 3H), 1.25-1.35 (m, 4H), 2.05 (m, 2H), 3.95 (d, J = 5Hz, 2H), 4.55 (s, 2H), 5.50-5.80 (m, 2H), 7.20-7.40 (m, 5H). MS (m/z): 204 (M^+, 5) 160 (7), 147(2), 107(12), 92(100).

Results and Discussion

Synthesis of benzyl propargyl ethers

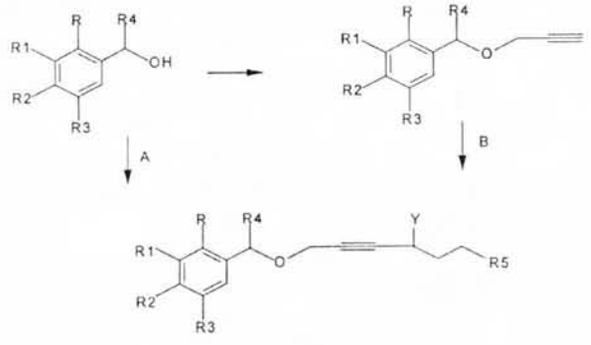
The strategy applied for the synthesis of mono/diaryl propargyl ethers and their analogues involved simple organic transformations. Etherification was achieved by treatment of alcohols prepared from corresponding ketones / aldehydes with propargyl bromide or benzyl bromides with propargyl alcohol under basic conditions. Accordingly, substituted benzyl alcohols were treated with propargyl bromide in presence of KOH/DMSO at room temperature (Scheme 1). Anion generated by abstraction of the acetylenic proton with *n*-butyl lithium at –78°C was quenched with *n*-butyraldehyde or *n*-butyl bromide (Scheme 1, B) to furnish the corresponding 1-benzyloxy-2-heptyn-4-ol or 1-benzyloxy-2-heptyne in good to excellent yield. Alternatively, substituted benzyl alcohol could also be condensed with 1-bromo 2-heptyne under alkaline conditions to furnish the required product. The second strategy (Scheme 1, A) proved to be easier and high yielding for the preparation of compounds listed in Table 1.

The diethers **1** to **7** were prepared by similar strategy, outlined in Scheme 2 from the corresponding dibromides for the aryl ethers or diol ethers.

Methyl 7-hydroxy-5-heptynoate was prepared by the reported method[20] starting from propargyl alcohol. Benzylation of methyl 7-hydroxy-5-heptenoate using benzyl bromide in the presence of sodium hydride at 0°C gave the corresponding ether **20** in fairly good yield. Under similar conditions *o*-oxylene dibromide when treated with two equivalents of methyl 7-hydroxy-5-heptanoate resulted in the formation of mono substituted products **21** and **22** with dehalogenation. However, under KOH/DMSO condition, mono substituted **23** was obtained leaving one benzyl bromide position unreacted.

Scheme 1

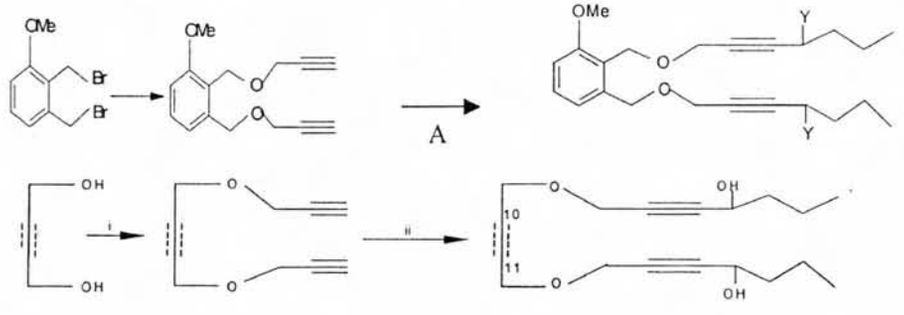
A=1- bromo-2-heptyne, B = n-butyraldehyde or n-bromobutane



Scheme 2

A = n-butyllithium, n-bromobutane or butyraldehyde

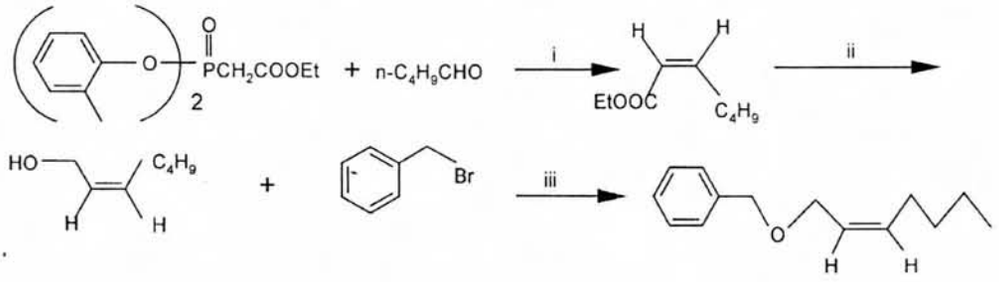
Reagents: KOH/DMSO, propargyl bromide, RT 3h, n-BuLi,C_4H_9Br or C_3H_7CHO, -78 °C



C_{10} – C_{11} = single bond, double bond, triple bond

Scheme 3

Reagents: i; NaH, THF, ii; -78°C, DIBAL-H, -78C, 2h, iii; NaH, THF, 0°C.



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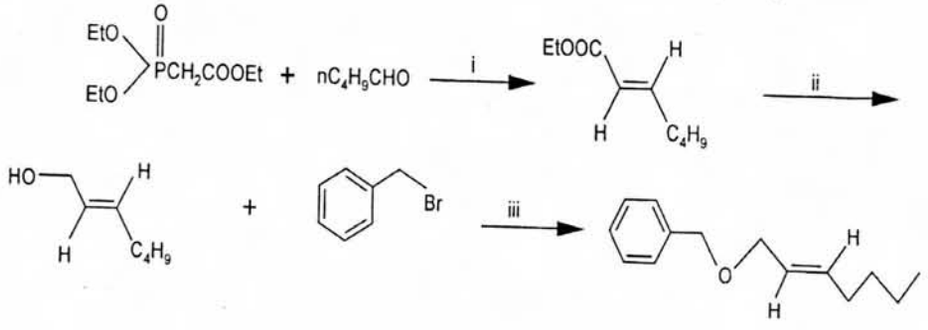
Scheme 4

Reagents: n-BuLi, THF 0°C: DIBALH, toluene, -78 °C: NaH, THF, 0 °C, 2h.



25

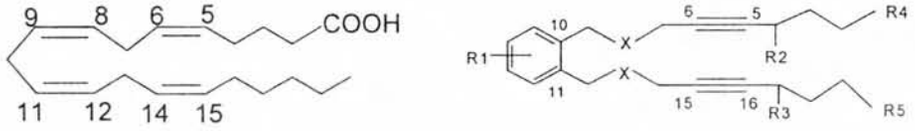
The *cis* isomers of **12** were prepared by modified Horner-Emmos[21] reaction using ethyl (di-*o*-toluoxyphophono) acetate (prepared from ethyl (dichlorophosphono) acetate and *o*-cresol). Reaction of *n*-pentanal with this reagent (Scheme 3) under controlled temperature at –78°C in presence of sodium hydride furnished pure ethyl 2-(Z)-heptenoate in excellent yield. DIBAL-H reduction of this ester furnished *cis* alcohol, which on benzylation with benzyl bromide in presence of sodium hydride at 0°C furnished 1-[(Z)-2-heptenyloxymethyl] benzene **24**.

Similarly reaction of *n*-pentanal with triethylphosphonoacetate (Scheme 4) in the presence of *n*-BuLi at 0°C gave ethyl-2 (E)-heptanoate in almost pure form, which on similar sequence of reaction gave trans product **25** in excellent yield.

5-LOX inhibitory studies

Since 5-LOX enzyme attacks at 5 positions thereby shifting 5-6 double bond to 6-7 carbon atoms and subsequent removal of 7-hydrogen, it was planned to increase the reactivity of hydrogen atom at adjacent position. Double bond at C_{11}-C_{12} of arachidonic acid was blocked by a phenyl ring (Fig. 2). Model compounds were prepared for bio-evaluation with different functional groups as shown in Fig. 3 (1 to 7). Symmetry of both terminals (or two halves) of these molecules was expected to exhibit inhibition due to enzymatic oxidation on both sides. However, surprisingly the compounds **1** to **7** have no effect on 5-LOX enzyme activity whereas the monoether **8** exhibited a very weak inhibition. In order to improve the inhibition of mono-ether of type **8** other analogues **9** to **23** shown in Table 1 were synthesized and studied for their inhibitory potency.

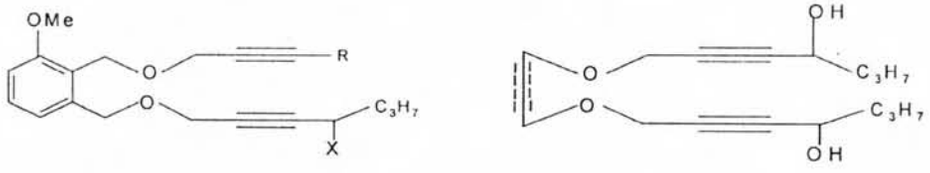
Screening of these analogues for 5-LOX inhibition showed maximum inhibition (IC_{50} 1.2 μM) for 1-(2-heptynyloxymethyl) benzene **12**. Addition of carboxyl group at the end of the alkyl side chain (R7) attached



I

R1 = H or Me or Ome
X = O or S or N-Me
R2, R3 = H or OH
R4, R5 = CO_2H or CO_2Me or Me
C_5-C_6 and C_{15}-C_{16} = double or triple bond

Fig. 2—Designated molecules based on arachidonic acid structure



1. R = C_4H_9, X = H
2. R = H, X = H
3. R = H, X = OH
4. R= HC-C_3H_7, X = OH (OH)
5. $C_{10}-C_{11}$ = single bond
6. $C_{10}-C_{11}$ = double bond
7. $C_{10}-C_{11}$ = triple bond

Fig. 3—Model compounds prepared

to the acetylenic group (**20**) abolished the inhibition. Introduction of sulphur in place of oxygen at position 'x' on parent compound (Fig. 1) (**19**) also reduced the inhibition (IC_{50} 64 μM). Introduction of hydroxyl group at position 'y' of the compound **12** (**8**) reduced the inhibition of 5-LOX drastically (IC_{50} 760 μM). Changing of the group at R_3 of the benzyl ring to $-CH_3$ **9** reduced the inhibition (IC_{50} 45 μM), whereas changing the group at R_3 to OCH_3 **10** or –Cl **11** totally abolished the inhibition. Selective reduction of the acetylenic bond of compound **12** to the *cis* (**24**) or *trans* (**25**) double bond resulted in the reduced inhibition. The *cis* isomer (**24**), however, was 20 times more potent than the *trans* isomer (**25**). The *trans* geometry does not mimic arachidonic acid and hence cannot act as a suicidal substrate as effective as *cis* isomer 1-(2-heptynyloxymethyl) benzene (**24**). Based on the lead obtained with potato 5-LOX for their inhibitory potential these compounds were tested against rabbit reticulocyte 15-LOX, which plays a key role in primary stage of atheroma formation[6]. The IC_{50} values for these compounds (**12**, **24** and **25**) are 61, 80 and 693 μM respectively. These studies reveal higher preference (> 50 fold) of compound **12** towards 5-LOX inhibition compared to that of rabbit reticulocyte 15-LOX. From the present study it is evident that compound **12** exhibits higher specificity towards 5 LOX inhibition. Further work directed towards kinetics of inhibition will be required to elucidate the nature of interactions of compound **12** with the 5- and 15- LOXs.

Summary and Conclusion

In an attempt to develop a new series of potent LOX inhibitors, we have designed and synthesized a series of benzyl propargyl ethers and they were tested against potato 5-LOX. Amongst the prepared compounds 1-(2-heptynyloxymethyl) benzene **12** was found to be the most effective against 5-LOX (1.2

μM). This compound, however, showed several fold lower inhibition towards rabbit reticulocyte 15-LOX (IC_{50} 61 μM).

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Patents

1 A process for the preparation of substituted 2-heptyne, 1-(arylmethoxy) B R D Wakharkar and N B Barhate, Indian Pat. Application No. 3072/DEL/1998
2 A process for the preparation of substituted 2-heptyne, 4-ol-1-(arylmethoxy) R D Wakharkar and N B Barhate, Indian Pat. Application No. 1564/DEL/1999