6-Aryl-4-Oxohexanoic Acids: Synthesis, Effects on Eicosanoid Biosynthesis, and Anti-Inflammatory *In Vivo*-Activities

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Abstract: The synthesis of a series of 6-aryl-4-oxohexanoic acids is described: This involves condensation of an appropriate aldehyde (**Ia-f**) and levulenic acid using catalytic amounts of piperidine and acetic acid in toluene to afford the 6-aryl-4-oxohex-5-enoic acids (**IIa-f**).The arylidene derivatives (**IIa-d**) were reduced by hydrogen at room temperature using palladium (10 %/carbon) as catalyst to produce 6-aryl-4-oxohexanoic acids (**IIIa-d**) as target compounds. In certain instances, the lactone derivative (**IVd**) was obtained as a low-melting by-product. These compounds were tested in two models used for evaluating the activity of non-steroidal anti-inflammatory drugs (NSAIDs). The first test is the effect of the synthesized compounds on arachidonic acid metabolism *in vitro* using human whole blood assay. The second is the *in vivo* carrageenan induced rat paw edema test. Compound **IIe** showed higher *in vivo*-activity compared to fenbufen at the same dose level (50mg/kg).

Key Words: 6-Aryl-4-oxohexanoic acids, Inflammation, NSAIDs, rat paw edema test, 5-lipoxgenase, arachidonic acid metabolism.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a widely prescribed and applied class of medications with established anti-inflammatory, analgesic, and antipyretic properties [1,2]. Despite these benefits, traditional NSAIDs such as (2-acetyloxy)benzoic acid, propionic or acetic acid derivatives have significant gastrointestinal (GI) toxicity as they inhibit both COX-1 and COX-2 isoforms and frequently cause gastrointestinal irritations or excessive bleeding [3-5]. These negative side effects prompted the development of selective COX-2 inhibitors as promising gastroprotective agents [6,7]. Many of these agents have been developed and marketed such as celecoxib and refecoxib, which have been extensively studied and showed exceptional anti-inflammatory properties with reduced GI toxicity. Later on, some potential limitations of long term certain COX-2 inhibitor therapy were described including ulcer exacerbation in high-risk patients, delayed gastroduodenal ulcer healing, thrombosis due to prostacyclin deficiency and kidney toxicity. Thus COX-2 inhibitors have not eliminated the need for improved drugs in the NSAID area [8-11].

In an important report [12], the effect of some selective COX-2 inhibitors and some dual (COX-1 and COX-2) inhibitors on carrageenan pleurisy in the rat over a time period ranging from 0 to 48 h after injection of the irritant was studied. The result of this study showed that the COX-2 inhibitors confered anti-inflammatory activity early in the inflammatory response, coincident with the expression of COX-2 protein as did the older dual inhibitors such as indomethacin. However, by 6 h the COX-2 inhibitors were without effect although the dual inhibitors still showed efficacy. At this point the COX-2 protein as shown by Western blotting was no longer present. This study showed the supremacy of conventional NSAIDs over the COX-2 inhibitors for chronic use. Moreover, withdrawal of rofecoxib from market recently by its originators due to adverse cardiovascular effects puts a question mark on the safety profile of other COXIBs in the long-term therapy.

Attempts to develop alternative COX-2 inhibitors by slight modification of the classical NSAIDs, resulted in discovery of lumiracoxib [13], a close structural analog to the well-known NSAID, diclofenac. Recently, a new approach of anti-inflammatory therapy constitutes the use of inhibitors of COX-1/2 and LOX pathways as an attempt to develop new active leads which may provide novel NSAIDs [14-17].

RATIONAL AND DESIGN

In the present work, and in continuing efforts to find distinctive structural template to NSAIDs with a wider safety margin, we reinvestigated many of the traditional carboxylic acid NSAIDs in order to design modified new leads endowed with possible potent and dual COX-1/2 and LOX inhibitory activity. We utilized the γ-oxobutanoic acid structural moiety with suitable substitution at the phenyl ring as structural template to the leads, since it was reported earlier, that arylhexenoic acids possess only weak anti-inflammatory activity in paw oedema test. Therefore, we became interested in synthesizing modified congeners in attempts to improve their activity [18]. To accomplish this goal, we designed a novel series of compounds with aliphatic carboxylic acid side chain in which 2 carbon spacer linker was used as a connection unit between the ketonic group of oxobutanoic acid and the aryl group mimicking certain NSAIDs such as fenbufen [19], bucloxic acid [20], and trepibutone [21], respectively. This linker could be an ethylene as in case or

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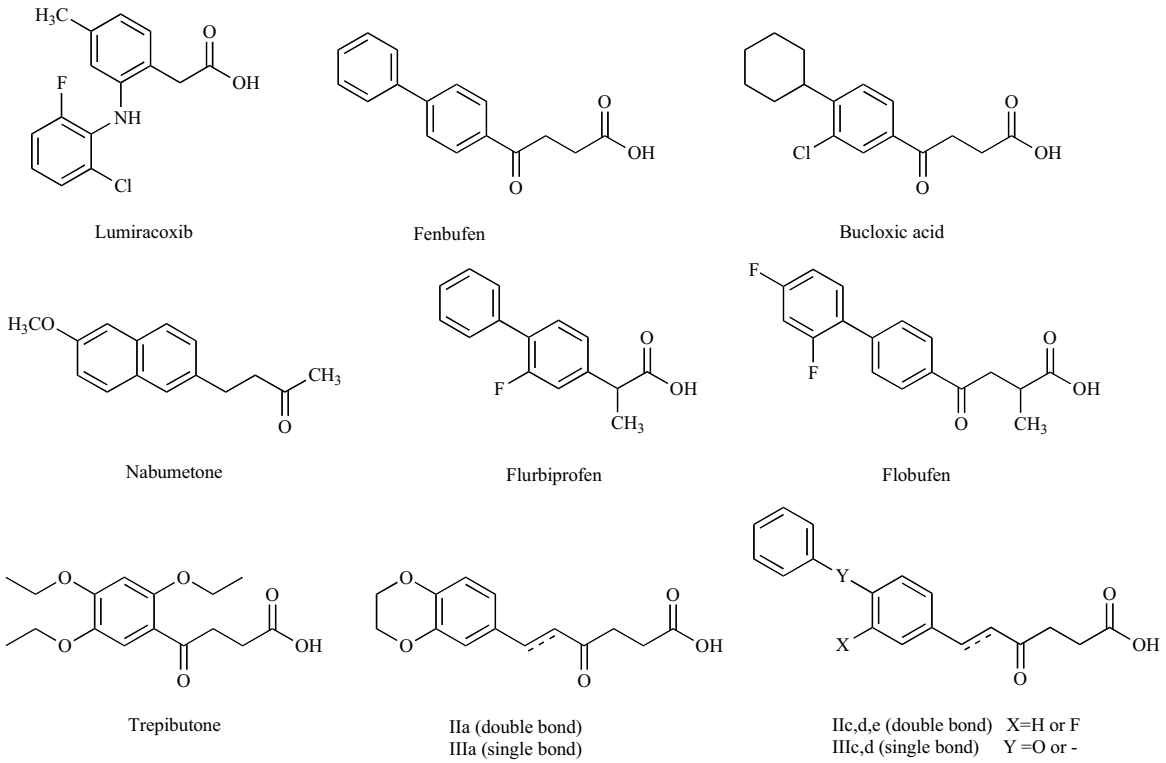
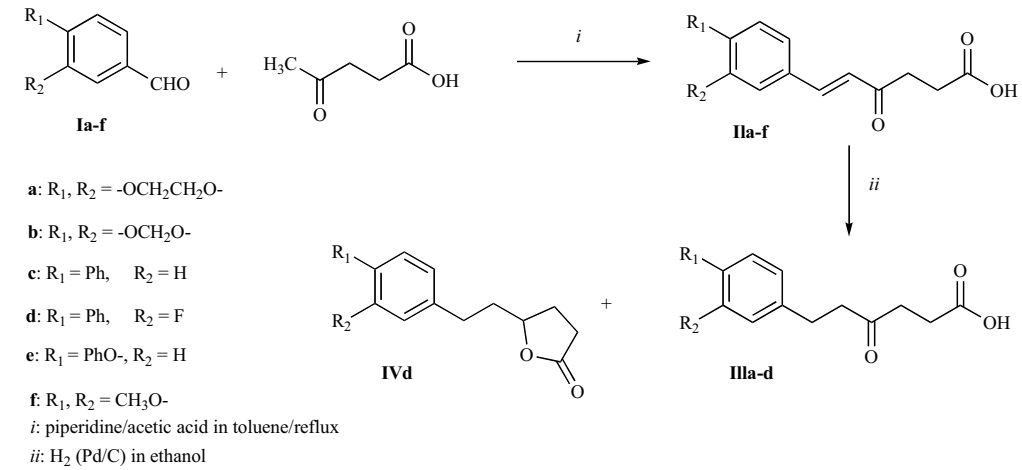


Fig. (1). Different NSAIDs serving as lead structures and target structures derived from these compounds.

alternatively an ethenyl group. This design may lead to prodrugs which could be vulnerable to metabolic enzymes *in vivo* to give the acetic acid derivative as in case of 6-methoxy-2-naphthylacetic acid (6-MNA), i. e. the active metabolite of nabumetone [22] (or biphenyl acetic acid out of fenbufen). In all compounds designed, we kept the oxobutanoic acid side chain as pharmacophoric moiety. On the other hand, the aromatic portion of the designed compounds is derived from known NSAIDs such as flurbiprofen and fenbufen [23], which both possess a biphenyl moiety. This prodrug approach could lead to improvement of pharmacokinetics and bioavailabilty of these compounds.

CHEMISTRY

Compound **IIb** was reported earlier by the first author [24], while **IIf** was synthesized in the literature applying a Wittig-Emmons reaction [25]. For the synthesis of the target compounds **III**, the following straightforward pathway was pursued: This involved condensation of appropriate aldehydes **Ia-f** and levulenic acid using catalytic amounts of



Scheme (1). Synthesis of 6-aryl-4-oxohexanoic acids (**IIIa-d**) by condensation of levulenic acid with aldehydes followed by hydrogenation. Further reduction of the carbonyl group lead to some lactone formation (**IVd**).

piperidine and acetic acid in toluene under reflux, with azeotropic removal of water using a Dean-Stark trap to give hexenoic acid derivatives **IIa-f**. Under these conditions, the carbanion formed at the α-methyl group of levulenic acid adds to the aldehyde carbon followed by dehydration to afford the arylidine keto acid derivatives **IIa-f**. ^{1}H-NMR shows the formation of *E*-isomers with minor amounts of *Z*-isomers, which are detected by TLC as minor spots that are removed upon recrystallization from the given solvent. The arylidene derivatives **IIa-d** were reduced at room temperature using 10 % palladium carbon as catalyst to afford compounds **IIIa-d**. In certain instance, a lactone derivative was obtained as a low-melting by-product, which was isolated by column chromatography and characterized by NMR and GC/MS. The formation of compound **IVd** can easily be explained by additional reduction of the carbonyl group to a hydroxy group, which reacted with the carboxy moiety.

PHARMACOLOGY

Cyclooxygenase and lipoxygenase isoforms are important enzymes of arachidonic acid metabolism, which is involved in formation of inflammatory eicosanoids. Activity of the enzymes was determined by quantification of their metabolites. Effects of synthesized compounds on COX-1 was controlled using 12-hydroxy-5,8,10-heptatrienoic acid (12-HHT) whereas formation of 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B_4 (LTB_4) marks activity of 5-LOX. Additionally, 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE) were analyzed as main metabolites of 12-LOX and 15-LOX pathway. Eicosanoid biosynthesis was monitored in human whole blood using HPLC method with UV-detection [14].

Table (**1**) shows that compounds **IIa-f** and **IIIa-d** failed to give significant activity on enzymes compared to control. Inhibition of eicosanoid formation was found less than 50 % at concentration of test compound of 10 μM in each case. Reference drug flurbiprofen demonstrated COX-1 inhibition with an IC_{50} of 0.17 μM relating to 12-HHT formation.

Selected compounds were further examined in an *in vivo*-inflammation assay: Intraplantar injection of carrageenan to rats resulted in severe discernible inflammation and significant increase in the mean volume of the challenged paw compared to that of the untreated paws. The increase in rat paws was most severe in control animals (45.2 % increase). The maximum oedema response was obtained 2 h following induction of inflammation, after which the increase in volume declined gradually until animals became virtually normal at the 12 h time point. Table (**3**) shows that fenbufen, **IIa**,**c**,**d**,**e**, and **IIIa** demonstrate decreased paw volume by a percent ranging from 0.3 to 25.8 (see experimental section). In the group corresponding to compound **IIIc** the treatment failed to demonstrate any significant inhibition from the control. The inhibition was maximum at the 2 h time point. In groups corresponding to compounds **IIa**, **IIc**, and **IIe** (see experimental section), the inhibition of paw volume increase started from the first time point and the paw volumes remained lower than control at all subsequent data points.

Despite the lack of *in vitro* activity of these compounds, many of them exhibited activity comparable to fenbufen in anti-inflammatory carrageenan induced rat paw oedema test especially compound **IIe**, which showed higher activity compared with fenbufen after 2 hours. These results might be attributed to conversion of these compounds to their active metabolites -probably acetic acid derivatives- *in vivo*.

EXPERIMENTAL SECTION

Chemistry

All reagents and solvents were purchased from Aldrich or Merck. Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H-NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz). Elemental analyses were performed on a Hereaus Vario EL apparatus. All new compounds were within ± 0.35 of the theoretical values. TLC was performed on silica gel F254 plates (Merck).

Table 1. Inhibition of Eicosanoid Formation (in %) Related to Control by Investigated Compounds at a Concentration of 10 μM, Three Determinations Per Compound

Compound	LTB_4	5-HETE	12-HETE	15-HETE	12-HHT
IIa	2 ± 10	3 ± 15	17 ± 16	27 ± 23	21 ± 7
IIb	n.i.	10 ± 26	n.i.	n.i.	n.i.
IIc	n.i.	16 ± 20	11 ± 12	3 ± 15	12 ± 6
IId	n.i.	14 ± 12	13 ± 9	15 ± 13	n.i.
IIe	n.i.	n.i.	8 ± 3	n.i.	14 ± 15
IIf	n.i.	18 ± 18	n.i.	n.i.	n.i.
IIIa	n.i.	10 ± 13	n.i.	n.i.	17 ± 9
IIIb	n.i.	n.i.	n.i.	n.i.	n.i.
IIIc	n.i.	n.i.	n.i.	n.i.	n.i.
IIId	n.i.	n.i.	5 ± 24	n.i.	n.i.

n.i. = no inhibition.

Table 2. Changes in Hind Paw Oedema Volume at Different Time Intervals after Treatment with a Single Dose (50 mg/kg) Orally of Fenbufen or Test Compounds in Carrageenan Induced Inflammed Rats (Means 5 Animals ± SEM)

Compound	Volume of Hind Paw Oedema			
	1h	2h	3h	6h
Control (saline)	4.19±0.12	4.37 ± 0.11	4.30 ± 0.08	4.11 ± 0.12
Fenbufen	4.15±0.11	3.50 ± 0.02	3.23 ± 0.09	3.06 ± 0.07
IIa	3.55±0.06	3.59 ± 0.10	3.27 ± 0.13	3.05 ± 0.03
IIc	3.39±0.08	3.66 ± 0.09	3.43 ± 0.09	3.24 ± 0.01
IId	4.15±0.09	3.78 ± 0.10	3.50 ± 0.02	3.35 ± 0.09
IIe	3.70±0.07	3.27 ± 0.09	3.27 ± 0.10	3.36 ± 0.08
IIIa	4.15±0.10	3.76 ± 0.12	3.80 ± 0.08	3.58 ± 0.09
IIIc	3.59±0.04	4.59 ± 0.04	4.62 ± 0.11	4.52 ± 0.10

All compounds are statistically significant different from control at $P < 0.05$.

For detection iodine vapour or UV light (254 nm), respectively, was used. MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Silica gel column chromatography utilized silica gel 60 63-200 μm (Baker).

General Procedure for the Synthesis of (5*E*)-6-(Substituted)-4-oxohex-5-enoic Acids (IIa-f)

Both the respective aldehyde (30 mmol) and levulinic acid (30 mmol) were dissolved in toluene (100 mL) containing acetic acid (3 mL) and piperidine (1 mL). The solution was heated under reflux using Dean-Stark water trap under nitrogen until the theoretical amount of water had been collected (~6 hr) and TLC analysis (7% methanol/$CHCl_3$) indicated disappearance of the starting material. The solvent was evaporated *in vacuo* and after cooling the solid product was washed twice with 10 mL of diethyl ether and then twice with 15 mL of 2 M HCl, dried and recrystallized from the appropriate solvent (given below).

(5*E*)-6-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-oxohex-5-enoic Acid (IIa)

Dark yellow pellets (recrystallized from toluene); m.p.: 138 °C; Yield: 78 %; ^{1}H-NMR ($CDCl_3$): δ 2.66 (t, 2H, *J*=7.2, CH_2CO_2H), 2.91 (t, 2H, *J* = 7.2, 2H CH_2-C(O)), 4.2 (s, 4H, -OCH_2CH_2O) 6.5-6.57 (d, 1H, *J* = 16.13, CH=CH-CO), 6.88 (d, 1H, *J* = 6.33, H-arom at C5), 7.03 (d, 1H, *J* = 7.4 aromatic H), 7.07 (s, 1H, aromatic H), 7.38-7.44 (d, 1H, *J* = 15 H, CO-CH=CH-); ^{13}C-NMR ($CDCl_3$) δ 28.01 (C2 aliphatic), 34.92, (C3 aliphatic), 64.16, 64.56 (C2, C3 dioxane), 116.93, 117.76, 122.41, 123.99, 127.93, 142.86, 143.75, 145.9, 178.1 (COOH), 197.76 (C=O); IR (KBr) γ (cm^{-1}): 1600, 1620 (C=C), 1675 (C=O), 1736 (COOH), 3275.

(*E*)-6-(Benzo[*d*][1,3]dioxol-5-yl)-4-oxohex-5-enoic Acid (IIb) [24]

Yellow crystals (from benzene); m.p.: 147 °C; Yield: 72 %; ^{1}H-NMR (DMSO-d_6): δ 2.67 (t, 2H, *J* = 7.2, CH_2CO_2H), 2.92 (t, 2H, *J* = 7.5, CH_2), 5.95 (s, 2H, O-CH_2-O), 6.50-6.56

Table 3. Inhibition (%) of the Rat Paw Oedema Compared to the Control at Different Time Intervals, after Treatment with a Single Dose (50 mg/kg) Orally of Fenbufen or Test Compounds

Compound	Time intervals			
	1h	2h	3h	6h
Fenbufen	0.85	19.80	24.86	25.54
IIa	15.35	17.90	23.94	25.84
IIc	19.15	16.29	20.30	21.06
IId	0.85	13.41	18.49	18.42
IIe	11.64	25.08	24.06	18.21
IIIa	0.85	14.00	11.69	13.01
IIIc	14.26	1.14	0.30	1.38

(d, 1H, *J* = 15, =CH-CO), 6.78-6.81 (d, 1H, *J* = 7.5, arom-H), 6.97-7 (d, 1H, *J* = 7.5 H , arom-H at C6), 7.01 (s, 1H, arom-H at C2), 7.38-7.44 (d, 1H, *J* = 15, H, CO-CH=CH-); IR (KBr) γ (cm^{-1}): 1600, (C=C), 1665 (C=O, ketonic), 1708 (COOH), 2910, 3200.

(*E*)-6-Biphenyl-4-yl-4-oxohex-5-enoic Acid (IIc)

Yellowish crystalline solid (from isopropanol); m.p.: 190 °C. Yield: 65 %; ^{1}H-NMR (DMSO-d_6): δ 2.51 (t, 2H, *J* = 7.2, CH_2CO_2H), 2.95 (t, 2H, *J* = 7.2, 2H CH_2-C(O)), 6.90-6.97 (d, 1H, J = 16.3, =CH-CO), 7.35-7.5 (m, 3H, arom), 7.63-7.7 (d, 1H, *J* = 16.3, H, CO-CH=CH), 7.71-7.81 (m, 6H, arom); ^{13}C-NMR ($CDCl_3$) δ 27.73 (C2 aliphatic), 34.86, (C3 aliphatic), 126.12, 126.62 (2C), 127.04 (2C), 127.91, 128.97 (2C), 129.02, 129.02, 133.47, 139.11, 141.5, 141.83, 173.75 (COOH), 198.32 (C=O); IR (KBr) γ (cm^{-1}): 1628, (C=C), 1684 (C=O, ketonic), 1713 (COOH), 2910, 3033.

(*E*)-6-(2-Fluorobiphenyl-4-yl)-4-oxohex-5-enoic Acid (IId)

Yellowish shiny crystals (from isopropanol). m.p.: 164 °C. Yield: (68 %); ^{1}H-NMR ($CDCl_3$): δ 2.77 (t, 2H, *J* = 7.2, CH_2CO_2H), 3.04 (t, 2H, *J* = 7.2, CH_2-C(O)), 6.56-6.75 (d, 1H, *J* = 16, =CH-CO), 7.36-7.61 (m, 5H, aromatic, 1H, =CH); IR (KBr) γ (cm^{-1}): 1625, (C=C), 1665 (C=O, ketonic), 1708 (COOH), 2922, 3010.

(*E*)-4-Oxo-6-(4-phenoxyphenyl)hex-5-enoic Acid (IIe)

Faint yellow crystals (from ether/petroleum ether). m.p.: 96 °C. Yield 67 %; ^{1}H-NMR ($CDCl_3$): δ 2.75 (t, 2H, *J* = 7.2, CH_2CO_2H), 3.01 (t, 2H, *J* = 7.2, CH_2-C(O)), 6.65-6.71 (d, 1H, J = 16, =CH-CO), 6.97 (d, 2H, *J* = 7.5, aromatic), 7.04 (d, 2H, *J* = 7.5, aromatic), 7.17 (t, 1H, *J* = 7, aromatic), 7.38 (t, 2H, *J* = 7, aromatic), 7.5 (d, 2H, *J* = 8.5, aromatic); ^{13}C-NMR ($CDCl_3$): δ 27.9 (C2 aliphatic), 34.96, (C3 aliphatic), 118.37, 119.76, 124.21, 124.38, 128.93, 129.94, 130.06, 142.51, 155.94, 159.87, 177.59 (COOH), 197.77 (C=O); IR (KBr) γ (cm^{-1}): 1589, (C=C), 1653 (C=O, ketonic), 1700 (COOH), 2936, 3040.

General Procedure for the Synthesis of (5*E*)-6-(substituted)-4-oxohexanoic Acids (IIIa-d)

To a suspension of 10.7 mmol of the corresponding (5*E*)-6-(substituted)-4-oxohex-5-enoic acid (**IIa-d**) in methanol (50 mL) 300 mg of 10% Pd/C was added, the resultant mixture was hydrogenated at atmospheric pressure at room temperature for 2 h. The catalyst was filtered off, and the solvent was distilled *in vacuo* to obtain a white solid which was recrystallized or column chromatographied (for details see below).

6-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-oxohexanoic Acid (IIIa)

Purified by silica gel column chromatography using $CHCl_3$/methanol 8% as eluent. White solid; m.p.: 86 °C; Yield: 65 %; ^{1}H-NMR ($CDCl_3$): δ 2.56 (t, 2H, *J* = 5, CH_2), 2.63 (t, 2H, *J* = 5, CH_2), 2.66 (t, 2H, *J* = 5, CH_2), 2.74 (t, 2H, *J* = 5, CH_2), 4.15 (s, 4 H, -OCH_2CH_2O), 6.55 (m, 2H, aromatic), 6.7 (d, 1H, aromatic); IR (KBr) γ (cm^{-1}): 1589, (C=C), 1702 (C=O, ketonic), 1745 (COOH), 2932, 3270.

6-(1,3-Benzodioxol-5-yl)-4-oxohexanoic Acid (IIIb)

White cottony crystals (from $CHCl_3$/*n*-hexane); m.p.: 77°C. Yield: 85 %; ^{1}H-NMR ($CDCl_3$): δ 2.61 (t, 2H, *J* = 5, CH_2), 2.64 (m, 4H, $2CH_2$), 2.87 (t, 2H, *J* = 5, CH_2), 5.9 (s, 2H, -OCH_2O-), 6.6-6.7 (m, 3H, aromatic); ^{13}C NMR ($CDCl_3$) δ 27.49 (C2 aliphatic), 29.4, (C3 aliphatic), 37.02, (C3 aliphatic), 44.39, (C3 aliphatic), 100.8, 108.23, 108.75, 121.03, 134.59, 145.83, 147.62, 177.17 (COOH), 207.72 (C=O); IR (KBr) γ (cm^{-1}): 1589, (C=C), 1701 (C=O, ketonic), 1745 (COOH), 2932, 3270.

6-Biphenyl-4-yl-4-oxohexanoic Acid (IIIc)

Fluffy white solid (from $CHCl_3$/*n*-hexane); m.p.: 136 °C; Yield: 70 %; ^{1}H-NMR (DMSO-d_6): δ 2.4 (t, 2H, *J* = 7.5, CH_2), 2.67 (t, 2H, *J* = 7.5, CH_2), 2.80 (s, 4H, $2CH_2$), 7.26-7.6 (m, 7H, aromatic), 12.0 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6) δ 27.6 (C2 aliphatic), 28.54, (C3 aliphatic), 36.64 (C5 aliphatic), 42.95, (C6 aliphatic), 126.41 (2C), 126.5 (2C), 127.1, (2C), 128.75, 128.81, 137.72, 140, 140.4, 173.69 (COOH), 208.19 (C=O); IR (KBr) γ (cm^{-1}): 1600, (C=C), 1704 (C=O, ketonic), 1718 (COOH), 2940, 3030. MS (EI): m/z (% rel. Int.) = 282 (M^+), 167(100).

6-(2-Fluorobiphenyl-4-yl)-4-oxohexanoic Acid (IIId)

Purified by silica gel column chromatography using $CHCl_3$/methanol 8% as eluent. ^{1}H-NMR ($CDCl_3$) δ 2.68 (m, 4H, $2CH_2$), 2.83 (t, 2H, *J* = 5, CH_2), 2.96 (t, 2H, *J* = 5, $2CH_2$), 6.97-7.05 (m, 2H, aromatic) , 7.32-7.55 (m, 6H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 27.09 (C2 aliphatic), 28.38, (C3 aliphatic), 36.4 (C5 aliphatic), 43.08, (C6 aliphatic), 115.14, 115.5, 123.72, 126.93 (arom-C), 127.83, (arom-C), 128.75, 128.81, 130.09, 135.12, 141.83, 141.95, 157.13, 161.7 (C-F), 177.52 (COOH), 206.74 (C=O); IR (KBr) γ (cm^{-1}): 1624 (C=C), 1706 (C=O, ketonic), 1718 (COOH), 2940, 3033.

5-[2-(2-Fluorobiphenyl-4-yl)ethyl]dihydrofuran-2(3H)-one (IVd)

Purified as a by-product by silica gel column chromatography using $CHCl_3$/methanol 8% as eluent. Faint yellowish white semi-solid. Yield: 12 %; ^{1}H-NMR ($CDCl_3$) δ 1.84-1.99 (m, 3H, aliphatic), 2.27-2.34 (m, 1H, aliphatic), 2.49 (t, 2H, CH_2), 2.53-2.82 (m, 2H , CH_2), 4.38-4.49 (m, 1H , lactone), 6.9-7.0 (m, 2H, aromatic) , 7.28-7.48 (m, 6H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 27.01 (aliphatic), 27.79, (aliphatic), 30.17 (aliphatic), 36.07(C-aliphatic), 78.63 (lactone C), 114.79, 115.15, 123.48, 126.54 (arom-C), 127.43, (arom-C), 127.93, 129.72, 134.61, 141.22, 141.34, 160.69 (C-F), 175.96 (lactone C=O). MS (EI): m/z (% rel. Int.) = 284 (M^+), 185(100).

Pharmacology

Effects of Compounds IIa-f and IIIa-d on Eicosanoid Biosynthesis (In Vitro-Measurements)

In vitro-human whole blood assay was run according to reference [14]. Freshly drawn heparinized blood from healthy volunteers (3 mL per determination) was incubated at 37 °C for 30 min in the presence of test compounds dissolved in 6 μL DMSO (final concentration 10 μM) or of 6 μL vehicle without compounds for control. Eicosanoid formation was induced with Ca-ionophor A23187 (30 μM), arachidonic acid (10 μM), $CaCl_2$ and $MgCl_2$ (final concentra-

tion 0.5 mM each). The samples were incubated for 30 min at 37 °C. Enzyme reaction was stopped by addition of ice-cold MeOH (containing PGB_1 as internal standard for HPLC determination) before blood samples were kept for 90 min at –18 °C. After addition of 4 mL of water to each sample, centrifugation at 2000 g and solid-phase extraction of the eicosanoids by Bakerbond SPE C-18 cartridges followed. Metabolites (LTB_4, 15-HETE, 12-HETE, 5-HETE, 12-HHT) were quantified by HPLC analysis using RP-18 column, mobile phase MeOH/water/acetic acid (77/23/0.01 v/v/v), UV detection with diode array detector and calculation of peak areas. Data obtained as mean of three determinations. Significant differences between eicosanoid formation in whole blood with test compounds and the controls were analyzed using *t*-test. The level of significance was set to $P < 0.05$.

Effect of Test Compounds on Paw Volume in Carrageenan Induced Rat Paw-Oedema Model (In Vivo-Measurements)

Material and Methods

Animals were obtained from animal house, Ain Shams University, Cairo, Egypt. Fenbufen was purchased from Sigma company, Germany.

The animals were fastened, with only free access to water, 16 hours before the experiment. Forty five rats were equally divided into 9 groups assigned Latin numbers I to IX, where I corresponds to normal animals, II to control, III to standard group (fenbufen), IV to **IIc**, V to **IIe**, VI to **IIIc**, VII to **IId**, VIII to **IIb** and IX to **IIIb**. Groups I and II were given saline by intragastric tube, while groups III through IX received the corresponding drug as indicated above. The dose was 50 mg kg^{-1}. Dosing volume was kept constant (10 mL kg^{-1}) and was completed with saline when required. Thirty min after oral treatment, group I received 0.05 mL of saline, while groups II through IX received 0.05 mL of carrageenan (1% solution in saline) sc on the plantar surface of the right hind paw. The right hind paw volume was measured immediately after carrageenan injection by electrolyte solution displacement using UGO-BASILE 7140 plethysmometer (Comerio, Italy) [26]. The volume was measured again 1, 2, 3 and 6 hours after carrageenan injection and results recorded.

Statistical Analysis of Data

Data obtained from animal experiments were expressed as the mean standard error (± SEM). Statistical differences between treatment and control groups was determined using ANOVA test. Data with $P < 0.05$ value was considered to be significant.

Acute Toxicity

Animals employed in the carrageenan-induced paw oedema experiment were observed for 24 h. No mortality was observed at the end of the observation period.

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