SEARCH FOR LIPOXYGENASE INHIBITORS AND STUDY OF THEIR PHARMACOLOGICAL ACTIVITY

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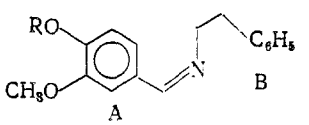
UDC 615.31:547.392.52].012.07

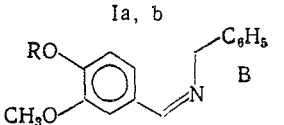
Monohydroxyeicosa-5,8,11,14-tetraenoic acids (HETE), such as 5-HETE, 12-HETE, and 15-HETE, appear as the first metabolites of arachidonic acid in mammalian tissues and play an important role in the regulation of intra- and intercellular communication [10]. The activation of their biosynthesis in the presence of lipoxygenase is part of the development of pathological processes [15], in consequence of which it is reasoned that inhibitors of lipoxygenase could be used as medicinal compounds for the treatment of inflammatory, allergic, and cardiovascular diseases [13, 15].

The goal of the present work consists of the investigation of new inhibitors of lipoxygenase and study of their effect on arterial pressure, thrombocyte aggregation, the proliferative activity of vascular system smooth muscle cells, and the level of cholesterol in atherosclerotic cells.

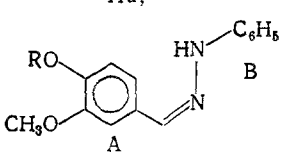
Known inhibitors of lipoxygenase appear to be either analogs of arachidonic acid or antioxidants containing an aromatic ring [7, 9, 10, 18]. We obtained a series of the simplest systems (I-III), containing the basic structural elements common to the many known inhibitors of lipoxygenase: two aromatic rings (A, B) separated from each other by three or four atoms, electron-donating substituents in positions 3 and 4 of ring A, π-electron systems conjugated with the aromatic ring, nitrogen atoms in positions β and γ to the benzene ring, and acidic functionality (phenolic hydroxyl) in the para-position.



Ia, b



IIa, b



IIIa, b

R = H (Ia, IIa, IIIa), CH_3 (Ib, IIb, IIIb)

The synthesis of the compounds was carried out in one stage — the condensation of aromatic aldehydes (vanillin and veratraldehyde) with aromatic amines (benzylamine, phenethylamine, and phenylhydrazine) resulting in the formation of the corresponding Schiff bases (I-III). Although compounds Ib-IIIb and IIIa are known [2, 6, 14], information on their physicochemical properties and their biological activities is limited [11, 12, 14].

Institute of Medical Radiology, Ministry of Health of the Armenian SSR. Institute of Experimental Cardiology, All-Union Cardiology Research Center, Academy of Medical Sciences of the USSR. Erevan Medical Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 9, pp. 1084-1088, September 1989. Original article submitted July 7, 1988.

0091-150X/89/2309-0749$12.50

TABLE 1. Influence of Compounds I-III on the Formation of 5-HETE, 12-HETE, 15-HETE, HHT, and TxB_2 (in % of control) in Incubations of Arachidonic Acid with Thrombocytes, Human Leucocytes, and Soybean Lipoxygenase (SBL)

Compound	Concentration, M	Thrombocytes			Leucocytes		SBL
		12-HETE	12-HHT	TxB_2	5-HETE	12-HETE	15-HETE
Ia	10^{-4}	102±10	106±11	105±8	54±20*	92±15	100
IIa	10^{-4}	97±10	98±12	92±11	112±12	123±18	100
IIIa	10^{-4}	17±6*	22±5*	3±2*	350±38*	12±8*	6,8±1,6*
	10^{-5}	18±8*	20±10*	7±3*	92±10	61±13*	18,0±1,5*
	10^{-6}	64±16*	50±12*	62±5*	88±12	74±13	36,0±2,0*
	10^{-7}	65±12*	50±12*	65±5*	38±15*	81±10	50,0±1,8*
	10^{-8}						66,3±2,1*
	10^{-9}						92,5±1,9
Ib	10^{-4}	103±10	102±12	102±14	48±22*	95±18	100
IIb	10^{-4}	96±12	97±13	96±11	155±11*	148±15*	100
IIIb	10^{-5}	18±6*	24±11*	10±7*	272±25*	21±10*	7,2±1,2*
	10^{-6}	47±12*	55±14*	60±9*	121±17	62±11*	20,3±2,1*
	10^{-7}	67±12*	55±15*	90±11	101±15	94±13	40,1±1,2*

*$p < 0.05$.

TABLE 2. Inhibition by Compounds I-III of Thrombocyte Aggregation Induced by Arachidonic Acid, Collagen, or ADF

Compound	EC_{50}, μM		
	ADF	arachidonic acid	collagen
Ia	0	—	870
IIa	1600	—	210
IIIa	310	98	8,7
Ib	0	—	0
IIb	0	—	2600
IIIb	560	97	23
Verapamil	—*	—	69

*A dash indicates study not conducted.

EXPERIMENTAL (CHEMISTRY)

N-(4-Hydroxy-3-methoxybenzylidene)phenethylamine (Ia). A mixture of 5 g vanillin (0.032 moles), 4.84 g phenethylamine (0.04 moles), 0.015 g p-toluenesulfonic acid, and 50 μl toluene was refluxed through a water trap until the water was completely removed. To the cooled solution 50 ml of water was added, and the precipitated crystals were washed with water. Yield 7.7 g (91.6%). Mp 111-113°C. R_f 0.67, chloroform/methanol 10:1 developed with iodine vapors. Mass spectrum m/z: 255 (M^+), 238 $[M - HO]^+$, 264 $[M - C_6H_5CH_2]^+$, 151, 137, 132, 122, 109, 104, 91, 77, 71, 57, 55. NMR spectrum (CD_3OD), δ, ppm: 3.1 (2H, d, J = 7 Hz, CH_2—C_6H_5), 4.86 (3H, d, J = 7 Hz, —CH_2—CH_2—C_6H_5), 3.91 (3H, s, CH_3—Ar), 6.8-7.2 (3H, m, CH of ring A), 7.3-7.6 (5H, m, CH of ring B), 8.1 (1H, s, HC=N). IR spectrum, ν_{max}, cm^{-1}: 1640 (C=N), 1550, 1480, 1460. UV spectrum (MeOH), λ_{max} (log ε): 272 (3.7976) — ring A, 307 (3.6726) — ring B, 396 (3.5464).

N-(4-Hydroxy-3-methoxybenzylidene)benzylamine (IIa). A mixture of 5 g vanillin (0.03 moles), 4.3 g benzylamine (0.04 moles), 0.015 g p-toluenesulfonic acid, and 50 ml toluene was refluxed through a water trap to dryness. On cooling the reaction mixture, crystals precipitated, which were filtered out and washed with water, dried,

and recrystallized from toluene. Yield 6.3 g of IIIa (79.7%). Mp 112-115°C. R_f 0.54, chloroform/methanol 12:1, developed with iodine vapors. Mass spectrum m/z: 241 (M^+), 266 [M — OH_3]$^+$, 264 [M — OH]$^+$, 210 [M — CH_3O]$^+$, 185, 164, 163, 150 [M — $CH_2C_6H_5$]$^+$, 148, 137, 117, 107, 106, 91. NMR spectrum (CD_3OD), δ, ppm: 3.88 (3H, s, CH_3OAr), 4.8 (2H, s, $CH_2C_6H_5$), 6.8-7.3 (3H, m, CH ring A), 7.4-7.6 (5H, m, C_6H_5 ring B), 8.2 (1H, m, HC=N). IR spectrum, ν_{max}, cm^{-1}: 1635 (C=N), 1595, 1590, 1520, 1515, 1500, 1465, 1450, 1445, 1430, 3085, 3028, 3010. UV spectrum (MeOH), λ_{max} (log ε): 275 (3.7941) — ring A, 307 (3.7149) — ring B, 398 (3.396).

Phenylhydrazone of 4-Hydroxy-3-methoxybenzaldehyde (IIIa). Mp 98-102°C, R_f 0.30, hexane/ether, 3:2, iodine vapors.

N-(3,4-Dimethoxybenzylidene)phenethylamine (Ib). R_f 0.67, chloroform/methanol, 12:1, iodine vapors.

N-(3,4-Dimethoxybenzylidene)benzylamine (IIb) Hydrochloride. Mp 98-100°C. R_f 0.77, hexane/ether 3:2, iodine vapors. Mass spectrum m/z: 255 (M^+), 240 [M—CH_3]$^+$, 224 [M—CH_3O]$^+$, 195, 194, 180, 179, 178, 166, 165, 152, 151, 137, 118, 117, 104, 91. NMR spectrum ($CDCl_3$), δ, ppm: 3.79 (3H, s, CH_3OAr), 3.84 (3H, s, CH_3OAr), 4.76 (2H, s, —$CH_2C_6H_5$), 6.6-7.1 (3H, m, CH ring A), 7.2-7.4 (5H, m, CH ring B), 8.2 (1H, m, HC=N). IR spectrum, ν_{max}, cm^{-1}: 1640 (C=N), 1598, 1585, 1518, 1498, 1460, 1445, 1420, 3065, 3030, 3015. UV spectrum (MeOH), λ_{max} (log ε): 268 (3.8421) — ring A, 314 (3.6576) — ring B.

Phenylhydrazone of 3,4-Dimethoxybenzaldehyde (IIIb). Mp 116-120°C. R_f 0.27 in hexane/ether 3:2, iodine vapors. Mass spectrum, m/z: 256 (M^+), 241 [M — CH_3]$^+$, 219, 128, 106, 93, 92, 91. NMR spectrum ($(CD_3)_2CO$): 3.76 (3H, s, CH_3OAr), 3.83 (3H, s, CH_3—OAr), 6.6-7.4 (8H, M, CH—Ar). 7.73 (1H, s, HN—Ar), 9.08 (1H, s, HC=N). IR spectrum, ν_{max}, cm^{-1}: 1600, 1575, 1518, 1495, 1465, 1450, 1440, 1410, 3290. UV spectrum (MeOH), λ_{max} (log ε): 309 (3.8348), 358 (4.0689). The composition of Ib, IIb and IIIb was confirmed by data from mass, NMR, IR, and UV spectra. Elemental analysis data corresponded satisfactorily with the calculated values.

EXPERIMENTAL (BIOLOGY)

The study of the influence of compounds II-III on the metabolism of [1-^{14}C]-arachidonic acid in human thrombocytes and granulocytes was carried out using high-performance liquid chromatography and autoradiographic thin-layer chromatography according to previously described methods [1, 17].

The activity of soybean lipoxygenase was determined spectrophotometrically [3]. The investigation of the influence of compounds I-III on arterial pressure was conducted on narcotized cats, recording the pressure in the femoral artery with an "Elema" sensor. The compounds were administered intravenously in 0.5 ml DMSO.

Analysis of the vacular effects of compounds I-III was carried out on isolated strips of the feline carotid artery with the "HF-Motel" sensor. In a series of experiments the serotonin antagonist methysergide and the alpha-adrenoreceptor blocker prazosin were used.

The study of the effects of compounds I-III on the aggregation of thrombocytes was conducted with thrombocyte-rich plasma and also with whole human blood, using the aggregometers "Chrono-Log" and "Payton." The aggregation inducers used included ADF (10^{-5} M), arachidonic acid (33 μM), and collagen (3.5 μg/ml). The DMSO content did not exceed 0.1%.

The investigation of the effects of compounds I-III on the proliferative activity of smooth muscle cells of vascular walls and the level of cholesterol in them was conducted according to previously described methods [16].

The study of the influence of compounds I-III on the activity of human thrombocyte lipoxygenase showed that phenylhydrazones IIIa and IIIb inhibited the biosynthesis of 12-HETE (EC_{50} about 1 μM). In addition, phenylhydrazones IIIa and IIIb significantly suppressed the biosynthesis of thromboxane B_2, probably due to inhibition of cyclooxygenase, the activity of which was judged by the formation of HHT 8 (Table 1).

These data correlate with the results of experiments on the effect of compounds I-III on thrombocyte aggregation presented in Table 2, in which it is apparent that the same phenylhydrazones IIIa and IIIb inhibit this aggregation, apparently in connection with their effects on the biosynthesis of thromboxane B_2. Furthermore, phenylhydrazones IIIa and IIIb correspondingly increase by 40 and 42% the effects of prostacyclins, inhibiting the ADF-induced aggregation of thrombocytes.

From the data presented in Table 3 it follows that phenylhydrazones IIIa and IIIb have no significant effect on feline arterial pressure or the tonus of isolated vasculature of these animals, while phenethylamines Ia and Ib and benzylamines IIa and IIb showed vasopressor action.

TABLE 3. Vasopressor Action of Compounds I-III and Noradrenaline on Narcotized Cats

Compound	EC_{50}, μg/kg	Compound	EC_{50}, μg/kg
Ia	42,4	Ib	31,9
IIa	790,0	IIb	641,8
IIIa	>2000,0	IIIb	1567,0
Noradrenaline	4,7		

TABLE 4. Effects of Compounds Ia-IIIa and IIIb on the Incorporation of [^{3}H]-Thymidine into DNA of Cultured Cells of Damaged Atherosclerotic Human Aorta, and Their Free and Bound Cholesterol Contents

Compound	MEC,* M	Thymidine incorporation		Cholesterol content	
		%	EC_{50}	%	EC_{50}
Control	—	100	—	0	—
Ia	10^{-4}	45,7±0,7	$1,0 \cdot 10^{-4} \pm 0,16$	13,0±1,6	$1,7 \cdot 10^{-4} \pm 0,41$
IIa	10^{-4}	60,1±1,6	$4,8 \cdot 10^{-4} \pm 0,75$	42,0±2,2	$1,2 \cdot 10^{-4} \pm 0,19$
IIIa	10^{-4}	55,7±0,5	$2,1 \cdot 10^{-4} \pm 0,62$	11,0±1,7	$>2,0 \cdot 10^{-4}$
IIIb	10^{-5}	60,0±0,9	$0,8 \cdot 10^{-4} \pm 0,26$	17,0±2,1	$0,61 \cdot 10^{-4} \pm 0,17$
Verapamil	$5 \cdot 10^{-5}$	13,4±0,9		23,4±1,9	—

*MEC — maximal effective concentration.

Experiments on isolated blood vessel segments showed that the vasopressor action of amines I and II was connected with a direct effect on the vessel walls. This effect varies in the series of compounds a and b directly with the hypertensive effect. Phenethylamines Ia and Ib exhibit vessel narrowing actions on strips of feline femoral arteries, equal in strength to 44 and 67% contraction induced by potassium depolarization. This vessel-contraction effect is eliminated by prazosin, which indicates the participation of α-adrenoreceptors in the vasotropic effect of phenethylamines Ia and Ib, but the effect is not altered by methysergide.

These results suggest a correlation between the structures of the compounds and their vascular-thrombocytic effects: a shortening of the carbon chain between the aromatic rings (I-II) leads to a decrease in affinity of the adrenaline analogs for the α-adrenoreceptors; substitution of a carbon atom by nitrogen (II-III), resulting in conjugation of the aromatic ring system with the p-electrons, brings about an inhibition of enzymes involved in the biosynthesis of eicosanoids and in the suppression of thrombocyte aggregation.

Analogously, compounds I-III have an effect on the biosynthesis of 15-HPETE by soybean lipoxygenase (see Table 1). It is, however, somewhat unexpected that compounds I-III display an effect on the biosynthesis of 5-HETE in human leucocytes (see Table 1). Phenylhydrazones IIIa and IIIb in small doses exhibit inhibitory effects but in large doses show stimulatory effects, while on the other hand the phenethylamines Ia and Ib inhibit the biosynthesis of 5-HETE in concentrations of 10^{-4} M.

Studies of the effects of compounds I-III on levels of cholesterol in isolated human aortal smooth muscle cells and the increase in proliferative activity they induce (vessels from patients who died of myocardial infarction showed that all the compounds studied showed antiatherosclerotic action (Table 4).

If the effect of the lipoxygenase inhibitor phenylhydrazones Ia, IIIb is interpreted in the terms presented above, then the antiatherogenic activities of benzylamine IIa and phenethylamines Ia and Ib are apparently not connected with the biosynthesis of 12- and 15-HETE. It is, however, not excluded that nonspecific activation of lipoxygenase can produce a favorable effect on angiohemic homeostasis, since the product of lipoxygenic oxidation of linolenic acid, 13-hydroxyoctadecadienoic acid, promotes adhesion of thrombocytes to the endothelium [4, 5]. It is possible that its accumulation in vascular strips is a compensatory reaction and thus activation of its biosynthesis by benzylamine IIa can have a protective role.

Thus, study of the biological activity of arylalkylamines I-III provides a useful approach to the search for new drugs that act on the various elements of circulatory homeostasis, and the study of the structure—activity relationships of the compounds enables one to obtain new synthetic compounds with high vasotropic and antithrombocytic activity.

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