J. Pharm. Pharmacol. 1998, 50: 583–591
Received December 9, 1997
Accepted February 18, 1998

Synthesis of Novel Derivatives of Aroylaminoalcohols and 3-Amino-substituted 1-Phenylpropanols with Potential Anti-inflammatory and Immunomodulating Activities

ANTONIS M. GAVALAS, LYGERI HADJIPETROU* AND PANOS N. KOUROUNAKIS

*Department of Pharmaceutical Chemistry, School of Pharmacy and *Department of Genetics, Development and Molecular Biology, School of Biology, Faculty of Sciences, Aristotelian University of Thessaloniki, Thessaloniki 540 06, Greece*

Abstract

The synthesis of aroylaminoalcohols and 3-amino-substituted 1-phenylpropanols is described. These novel basic compounds have potent anti-inflammatory activity, significantly inhibiting rat paw oedema induced by a variety of phlogistic agents as a result of the release of inflammatory mediators such as histamine, 5-hydroxytryptamine, kinins, prostaglandins or leukotrienes.

The biological activity of a selected, representative number of these compounds was examined on adjuvant-induced arthritis, a good animal model for rheumatoid arthritis in man. The results show that 3-(1-hydroxymethylpropylamino)-1-phenylpropan-1-one hydrochloride (**2**) and 3-(3-hydroxypiperidin-1-yl)-1-phenylpropan-1-one hydrochloride (**4**) effectively suppress the secondary lesions of adjuvant arthritis. 2-(3-Hydroxy-3-phenylpropylamino)-butan-1-ol hydrochloride (**6**) had no preventive activity in this animal model. In addition, several of the compounds suppressed the mitogenic responses of T-lymphocytes to concanavalin A, suggesting direct or indirect action on lymphocytes and therefore possible immunosuppressive properties. Finally, we investigated the in-vitro effects of compounds **2** and **4** on production of interleukins 1 and 2 (IL-1 and IL-2). We found that compound **4** suppressed the in-vitro production or action of IL-2 and IL-1. In contrast, compound **2** significantly increased the IL-1 activity of arthritic macrophages while reducing the ability of normal and arthritic splenocytes to produce or release IL-2.

Our data suggest that compounds **2** and **4** have immunomodulatory properties that influence T lymphocyte functions.

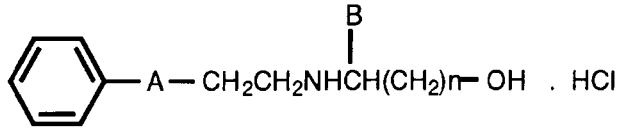
Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used clinically. However, despite their great number none is very effective therapeutically and almost all have several undesired, often serious, side effects (Calhoun et al 1995) and so long-term administration is not advisable and the need for new anti-inflammatory drugs is obvious.

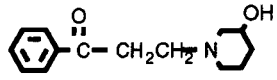
A series of novel derivatives of aroylaminoalcohols and 3-amino-substituted 1-phenylpropanols have been synthesized (compounds **1–6**; Table 1) in an attempt to find improved anti-inflammatory agents. One of the most interesting characteristic of these novel compounds is their basic nature, which differentiates them from the classical, acidic non-steroidal anti-inflammatory agents. It was of interest, therefore, to study in detail the anti-inflammatory and immunosuppressive properties of these novel compounds.

Correspondence: P. N. Kourounakis, Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Thessaloniki 540 06, Greece.

The anti-inflammatory properties of the compounds were evaluated by measuring their inhibitory effect on rat paw oedema induced by various phlogistic agents (Kalsi et al 1990; Gavalas et al 1991). To evaluate the possible immunomodulating activity of a number of these compounds, rats with adjuvant-induced arthritis were used. Adjuvant-induced arthritis is considered to be an animal model for rheumatoid arthritis in man and has been extensively studied not only as a cellular immune disease model but also to evaluate various anti-inflammatory and immunosuppresive drugs (Panosian et al 1986; Fawcett et al 1990). In previous studies various immunological parameters of arthritic rats have been found to be impaired: the

Table 1. Physicochemical characteristics and elemental analysis of the compounds.



	A	B	n	Retention factor (base)	Yield (%)	Mp (°C)	Recrystallization solvents	Elemental analysis					
								Calculated (%)			Found (%)		
								C	H	N	C	H	N
1	–C=O	–H	1	0·432	30	156–158	CH_3OH/CH_3COOEt	57·52	7·02	6·10	57·75	6·89	5·81
2	–C=O	CH_2CH_3	1	0·648	55	146–148	CH_3OH/CH_3COOEt	60·58	7·81	5·43	60·27	7·85	5·43
3	–C=O	–H	2	0·305	30	138–140	CH_3OH	59·13	7·44	5·75	58·74	7·43	5·63
4				0·794	77	176–178	$(CH_3)_2CHOH$	62·33	7·47	5·19	62·29	7·60	5·52
5	–CHOH	–H	1	0·611	89	130–131	C_2H_5OH/Et_2O	57·02	7·82	6·04	57·03	7·79	5·84
6	–CHOH	CH_2CH_3	1	0·364	93	119–120	C_2H_5OH/Et_2O	60·10	8·54	5·39	60·05	8·74	5·85

Silica gel TLC; mobile phase ammonia-saturated chloroform/ethanol (1 : 1).

response of splenic lymphocytes from arthritic rats to T cell mitogens is reduced during the active phase of the disease, owing to suppressor cells (Kourounakis et al 1991). Accordingly, it was interesting to evaluate the effect of treatment with selected compounds on some of the adjuvant-induced arthritis-impaired parameters.

In this paper, we also report the effect of compounds **2** and **4** on the production of interleukin 1 and 2 (IL-1 and IL-2), to elucidate, if possible, the mechanism of the anti-inflammatory and immunomodulating actions of these compounds. IL-1 and IL-2 are very important soluble mediators which participate in the development of adjuvant-induced arthritis (Lipsky et al 1989).

Materials and Methods

Materials

Melting points were taken on a Mel Temp II capillary apparatus. IR spectra were determined on a Shimadzu FTIR-8101 M Fourier transform infrared spectrophotometer. ^1H NMR spectra were recorded with a Brüker AW-80 spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 CHN elemental analyser. All chemical reagents were obtained from commercial sources. Thin-layer chromatography (TLC) was performed on silica gel.

Carrageenan type K 100 (Agno, Greece), Baker's dry yeast (Zanae, Greece), concanavalin A (Con-A; Sigma, St Louis, MO) and Nystatin (Mycostatin; Serva, Heidelberg, Germany), suspended in saline, were used as phlogistics. Indomethacin (Sigma) was used as reference compound. Freund's complete adjuvant was purchased from Difco (Detroit, MF). RPMI-1640 tissue culture medium and buffer solutions were purchased from Serva. Foetal calf serum was purchased from Gibco (Grand Island, USA). Tritiated thymidine was purchased from NEN (Boston, MA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide from Sigma.

Chemistry

(2-Hydroxyethylamino)-1-phenylpropan-1-one hydrochloride (1). Compound **1** was synthesized by the Mannich reaction (Tramontini 1973). Dry ethanolamine hydrochloride (13·7 g, 0·14 mol), powdered paraformaldehyde (4·3 g, 0·14 mol) and acetophenone (12·9 g, 0·11 mol) were introduced to a round-bottomed flask with a reflux condenser attached. Absolute ethanol (17 mL) containing concentrated HCl (0·5 mL) was added and the reaction mixture was heated under reflux for 3 h whereupon it became almost clear and homogenous (Figure 1). After 3 h the mixture was left to cool and stand overnight at room temperature. The crystals formed were collected and made alkaline (5 M NaOH) to furnish the free base. This was extracted with ether (3 × 100 mL) and the combined extracts were washed with saturated aqueous sodium chloride (1 × 100 mL), the ether layer was dried over K_2CO_3 and the solvent evaporated under reduced pressure. The hydrochloride salt of compound **1** was prepared in ethanol by addition of an ethereal solution of HCl and was recrystallized from a mixture of solvents (Table 1). ^1H NMR ($CDCl_3$): δ 2·4–3·2 (m, 6H, OH, NH, $2CH_2$), 3·2–

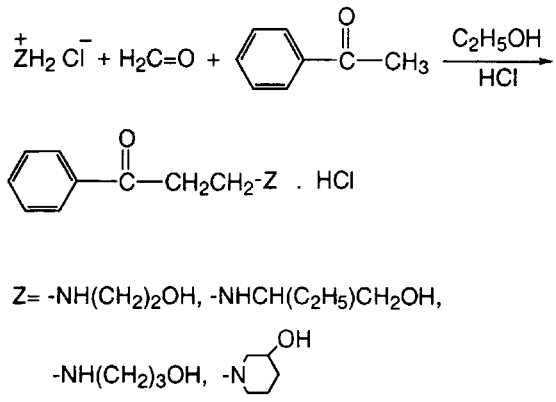


Figure 1. Synthetic pathway for the preparation of aroylaminoalcohols.

3·8 (m, 4H, $2CH_2$), 7·5–8·0 (m, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3300 (OH), 2750 (NH_2^+) 1680 (C=O).

(1-Hydroxymethylpropylamino)-1-phenylpropan-1-one hydrochloride (2). Compound **2** was prepared from 2-aminobutanol hydrochloride (15·4 g, 0·12 mol), powdered paraformaldehyde (3·8 g, 0·12 mol) and acetophenone (11·4 g, 0·10 mol) in the presence of absolute ethanol (17 mL) and concentrated HCl. The reaction and isolation procedure were as described for compound **1**: ^1H NMR ($CDCl_3$): δ 0·95 (s, 3H, CH_3), 1·2–1·6 (m, 2H, CH_2), 2·1–2·8 (m, 2H, CH_2), 3·2–3·8 (m, 7H, OH, NH, CH, $2CH_2$), 7·2–8·0 (m, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3300 (OH), 2750 (NH_2^+), 1680 (C=O).

(3-Hydroxypropylamino)-1-phenylpropan-1-one hydrochloride (3). Compound **3** was prepared from 3-aminopropanol hydrochloride (40 g, 0·36 mol), powdered paraformaldehyde (10·9 g, 0·36 mol) and acetophenone (32·9 g, 0·26 mol) in the presence of absolute ethanol (43 mL) and concentrated HCl. The reaction and isolation procedure were as described for compound **1**: ^1H NMR ($CDCl_3$): δ 1·6–1·9 (m, 2H, $2CH_2$), 2·5–3·2 (m, 6H, $2CH_2$, OH, NH), 3·3–3·7 (m, 4H, $2CH_2$), 7·2–8·0 (m, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3300 (OH), 2750 (NH_2^+) 1680 (C=O).

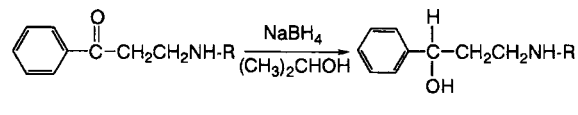
(3-Hydroxypiperidin-1-yl)-1-phenylpropan-1-one hydrochloride (4). Compound **4** was prepared from 3-hydroxypiperidine hydrochloride (19·2 g, 0·14 mol), powdered paraformaldehyde (4·3 g, 0·14 mol) and acetophenone (12·9 g, 0·11 mol) in the presence of absolute ethanol (17 mL) and concentrated HCl; the mixture was heated under reflux for 4 h. The reaction and isolation procedure were as described for compound **1**. ^1H NMR ($CDCl_3$): δ 1·3–1·7 (s, 4H, $2CH_2$), 2·3–2·9 (m, 7H, OH, $3CH_2$ of piperidine ring), 3·0–3·4 (t, 2H, C-4 of piperidine ring), 3·5–3·8 (s, 1H, C-3 of piperidine ring), 7·5–8·1 (m, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3200 (OH), 1680 (C=O).

(2-Hydroxyethylamino)-1-phenylpropan-1-ol hydrochloride (5). The free base of compound **1** (1·7 g, 9 mmol) was reduced to the corresponding alcohol by treatment with $NaBH_4$ (0·34 g, 9 mmol) in a mixture of isopropanol (45 mL) and water (18 mL) at room temperature. When addition of $NaBH_4$ was completed the mixture was stirred at room temperature for 24 h (Figure 2). The reaction solvents were then removed under reduced pressure and the residue suspended in water and extracted with ether (3 × 100 mL). The combined organic extracts were washed once with saturated aqueous sodium chloride (100 mL) and dried over anhydrous K_2CO_3. The solvent was evaporated under reduced pressure and the residue dissolved in ethanol and acidified with HCl to give the hydrochloride salt of compound **5**. ^1H NMR ($CDCl_3$): δ 1·2–1·6 (m, 2H, CH_2), 2·5–3·0 (m, 5H, NH, $2CH_2$), 3·1–3·5 (s, 2H, 2OH), 3·6–3·9 (q, 2H, CH_2), 4·6–5·0 (m, 1H, CH), 6·9–7·4 (m, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3400 (OH), 2750 (NH_2^+).

(3-Hydroxy-3-phenylpropylamino)-butan-1-ol hydrochloride (6). Compound **6** hydrochloride was prepared by reduction of the free base of compound **2** (2 g, 9 mmol) with $NaBH_4$ (0·34 g, 9 mmol) in a mixture of isopropanol (45 mL) and water (18 mL) as described for compound **5**. ^1H NMR ($CDCl_3$): δ 0·7–0·9 (m, 3H, CH_3), 1·2–1·9 (m, 4H, $2CH_2$), 2·3–3·0 (m, 3H, CH_2N, CHN), 3·2–4·0 (m, 5H, 2OH, NH, CH_2O), 4·8–5·0 (t, 1H, CHO), 7·0–7·4 (s, 5H, C_6H_5). IR (Nujol, cm^{-1}): 3400 (OH), 2700 (NH_2^+).

Biological experiments

Animals. The study was performed on highly inbred Fisher-344 rats, 2–4 months old. The animals, bred in our facilities, were housed under standard laboratory conditions. A diet of commercial food pellets and water were freely available.



R= -CH_2CH_2OH, -$CH(C_2H_5)CH_2OH$

Figure 2. Synthetic pathway for the preparation of 3-amino-substituted 1-phenylpropanols.

Phlogistics-induced oedema. Experiments were performed on groups of six rats, 160–180 g. A single dose of 0·2 mmol kg^{-1} of the test compound or 0·01 mmol kg^{-1} indomethacin were administered intraperitoneally 15 min before the induction of oedema. Control animals received only saline. The oedema was induced by injection of 1% carrageenan (0·1 mL) or 5% dry baker's yeast or 1% concanavalin A or 15 000 int. units nystatin, into the right hind foot paw, the left serving as the control. Thereafter, the animals of a particular group were killed and both hind paws were severed above the ankle joint and immediately weighed on a sensitive analytical balance. For each animal the swelling caused by the phlogistic is given as the percentage weight increase of the right hind paw in comparison with the untreated left hind paw; from this figure the percentage suppression of oedema in comparison with controls was also calculated. Statistical evaluation was performed by use of Student's *t*-test.

Induction of adjuvant arthritis and treatment with compounds ***2***, ***4*** *or* ***6.*** Groups of 5–10 rats were injected intradermally into the base of the tail with 0·1 mL Freund's complete adjuvant. Compounds **2**, **4** or **6** were administered intraperitoneally every day until the termination of the experiment. Rats were divided into four groups: groups 1 and 2 were injected with Freund's adjuvant, group 3 with the test compounds and group 4 with saline. In the preventive studies, 0·2 mmol kg^{-1} of compounds **2**, **4** and **6** were administered intraperitoneally to groups 2 and 3 once daily starting on the day after injection of the adjuvant and continuing until the termination of the experiment. For quantification of arthritis (arthritic score), a single point was assigned for each wrist or ankle area and an additional point was given for each involved phalangeal joint, up to maximum of 5 points per extremity (Kourounakis & Kapusta 1976; Kourounakis et al 1991).

Mitogenic response to concanavalin A. Spleens from normal or arthritic rats from untreated groups and groups treated with the test compounds were aseptically excised, pooled and appropriately prepared as cell suspensions in culture medium (RPMI complete, with 5% foetal calf serum). Cell viability was > 90% as determined by trypan blue exclusion. For mitogenic responses the cells were cultured in microtitre plates in the presence of suitable concentrations of concanavalin A (Con-A, 16 $\mu g\,mL^{-1}$) and different concentrations of the test compounds. Cultures were incubated at 37°C in 5% CO_2 for 72 h. Eighteen hours before the termination of the culture the mitogenic response to Con-A was evaluated by incorporation of [^{3}H]thymidine into the cells. The results are expressed as percentage of the value found in normal untreated animals (Kourounakis & Kapusta 1974).

Lymphokine production and assay. For production of interleukin 1 (IL-1), $5 \times 10^6\,mL^{-1}$ plastic adherent spleen cells from normal or arthritic rats were distributed in Linbro tissue culture dishes (24 wells) with 20 μL (25 $\mu g\,mL^{-1}$) of lipopolysaccharide (*Escherichia coli*) solution and incubated at 37°C in 5% CO_2. For IL-2 production spleen cells (6×10^6 cells mL^{-1}) from normal or arthritic rats were suspended in complete RPMI + 5% foetal calf serum with 16 $\mu g\,mL^{-1}$ Con-A (Yiangou & Hadjipetrou-Kourounakis 1989). Compounds **2** and **4** were added at the beginning of the culture, at concentration of 5 $\mu g\,mL^{-1}$, as preliminary experiments showed that this concentration was effective. The amounts of IL-1 and IL-2 produced were estimated by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye and measuring the optical density in an ELISA spectrophotometer (Mosmann 1983). The results are expressed as a percentage of the value found for normal untreated rats.

Results

Chemistry

The synthesized compounds, their physicochemical characteristics and elemental analysis are shown in Table 1.

Inhibitory effects of the synthesized compounds on phlogistics-induced oedema

All the compounds tested on carrageenan-induced oedema resulted in statistically significant anti-inflammatory activity during the incipient phase (1–2 h, data not shown) and during the second phase (4 h) of this inflammation. Indomethacin inhibited the carrageenan-induced oedema significantly only during the second phase. Compounds **2** and **4** had greater inhibitory effect, up to 66% and 67%, respectively, during the second phase of oedema (Table 2).

Compounds **1–4** inhibited oedema in both the early (2 h, data not shown) and late (4 h) phases of inflammation induced by Con-A. The greatest inhibitory activity was shown by compounds **3** and **4**, up to 61% and 58%, respectively (Table 2). The reduced derivatives of aroylaminoalcohols (compounds **5** and **6**) did not significantly inhibit this type of oedema (early or late phase), in contrast with indomethacin which had a significant anti-

Table 2. Effects of compounds on the oedema induced by various phlogistics, expressed as weight increase (%) of rat hind paw.

Compound	Increase of hind-paw oedema (%)							
	Carrageenan 4 h		Yeast 3 h		Nystatin 3 h		Concanavalin A 4 h	
Control	43·7 ± 2·8		50·0 ± 3·2		22·1 ± 3·5		35·6 ± 4·8	
1	27·4 ± 2·6	(37)	34·3 ± 3·7***	(32)	11·2 ± 0·9***	(49)	21·5 ± 3·3***	(40)
2	15·0 ± 3·8	(66)	18·1 ± 6·1***	(64)	11·0 ± 1·7***	(50)	19·8 ± 5·0***	(44)
3	22·3 ± 4·0	(49)	19·6 ± 3·5***	(61)	10·0 ± 2·3***	(55)	15·4 ± 4·1***	(57)
4	14·3 ± 4·8***	(67)	25·1 ± 5·8***	(50)	8·5 ± 3·6***	(62)	14·8 ± 4·3***	(58)
5	30·0 ± 3·8***	(31)	27·4 ± 2·8***	(41)	15·7 ± 2·9**	(29)	31·3 ± 3·9	(9)
6	28·8 ± 3·5***	(34)	22·6 ± 3·3***	(55)	14·3 ± 4·3**	(35)	32·5 ± 4·0	(9)
Indomethacin	23·1 ± 3·1***	(47)	33·8 ± 3·6***	(32)	11·4 ± 1·2***	(48)	19·2 ± 3·7**	(45)

Each value is the mean ± s.d. of results from three independent experiments with five animals in each group. $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the result for the control group. Values in parentheses represent inhibition (%) of oedema.

inflammatory effect in the late phase of Con-A oedema.

Subplantar injection of baker's yeast provoked rapid swelling of the paw to approximately 50% above the normal size. Three hours after treatment all compounds significantly reduced this oedema, in contrast with indomethacin which exerted a weak inhibitory effect (Table 2).

Nystatin-induced oedema was relatively long-lived, but the extent of swelling was small. All the examined compounds and indomethacin significantly inhibited the swelling 3 h after induction of nystatin oedema. Inhibition was more potent for the aroylaminoalcohol derivatives than for compounds **5** and **6** (Table 2).

Effect of compounds ***2***, ***4*** *and* ***6*** *on adjuvant-induced arthritis*

Treatment with compounds **2** and **4** effectively delayed and prevented the immune-mediated inflammation which developed after injection of Freund's adjuvant (Table 3). In contrast, treatment with compound **6** had a very weak suppressive effect in the chronic phase of adjuvant arthritis. Compared with the results obtained from arthritic controls, the arthritic score was reduced by 60–86% after treatment with compound **2** and almost completely suppressed after treatment with compound **4**.

Effects of the synthesized compounds on the mitogenic response of spleen cells to Con-A

The effect of in-vivo treatment with compounds **2** or **4** on the mitogenic response of splenocytes to Con-A in normal and arthritic rats, is shown in Table 4. The mitogenic response of spleen cells from 19 days post-adjuvant-injected rats was less than that of normal cells. We have previously found that the reduced mitogenic response of 14–19 days post-adjuvant-injected rat splenocytes is because of suppressor cells found in the spleens of these rats (Kourounakis et al 1991). Treatment of arthritic rats with these compounds did not improve this response. Furthermore, different concentrations of compounds **1–4** were added in-vitro to the cell cultures from normal and 19 days post-adjuvant-injected rats (Table 5). The addition of compound **1** did not affect the mitogenic response of normal spleen cells but it augmented the reduced mitogenic response of arthritic spleen cells. Compound **6** did

Table 3. Effects of treatment with compounds **2, 4** or **6** on the development and severity of adjuvant-induced arthritis.

Treatment	Arthritic score Days after adjuvant injection				
	11	13	15	17	19
Freund's complete adjuvant	1·6 ± 0·4	6·7 ± 1·7	11·0 ± 3·8	14·3 ± 3·2	15·8 ± 3·8
+ compound **2**	0	2·0 ± 1·8*	4·0 ± 1·3***	5·5 ± 1·6***	6·8 ± 3·5**
+ compound **4**	0	0	0	2·0 ± 0·2***	2·5 ± 0·5***
+ compound **6**	1·0 ± 0·6	3·0 ± 1·9	8·0 ± 2·5	9·4 ± 2·8	10·7 ± 2·6

Each value is the mean ± s.d. of the arthritic score (on day after injection of Freund's complete adjuvant) from five rats and is representative of values from two further independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the result from the untreated Freund's complete adjuvant group.

Table 4. Effect of in-vivo treatment with compounds **2** or **4** on mitogenic response (S.I.) to concanavalin A of splenocytes from normal and adjuvant arthritic rats.

Treatment	S.I.
Normal rats	92
Normal rats + compound **2**	67
Normal rats + compound **4**	49
Arthritic rats	53
Arthritic rats + compound **2**	21
Arthritic rats + compound **4**	30

Values are means of results from triplicate cultures. The results are representative of three similar experiments. S.I. = stimulation index = activity from cultures with mitogen /activity from cultures without mitogen.

not affect the mitogenic response of normal or arthritic spleen cells (results not shown). In contrast, the mitogenic response of arthritic spleen cells was suppressed in the presence of compounds **2**–**4**.

In-vitro effects of compounds 2 or 4 on lymphokine production and assay

The production of IL-1 from lipopolysaccharide-stimulated plastic adherent spleen cells from normal and 18 days post-adjuvant-injected rats, with or without the addition of compounds **2** and 4, is shown in Table 6.

We found that 18 days post-adjuvant-injected rat macrophages produced amounts of IL-1 almost equivalent to those synthesized by normal rat macrophages. When compounds **2** and **4** were added to normal macrophages during production of IL-1, smaller amounts of IL-1 were synthesized compared with controls. The addition of compound **4** during assay of IL-1 from arthritic-rat macrophages reduced the production of IL-1, in contrast with compound **2** which increased the amount produced.

Con-A-stimulated spleen cells of 18 days post-adjuvant-injected rats tended to produce smaller amounts of IL-2 than normal spleen cells (Table 6). In the presence of compounds **2** and **4** the ability of stimulated spleen cells from normal and 18 days post-adjuvant-injected rats to produce IL-2 was suppressed in comparison with controls.

Discussion

The aroylaminoalcohols were synthesized in good yields by use of the Mannich reaction, reaction of acetophenone with the appropriate aminoalcohol hydrochloride and paraformaldehyde in the presence of concentrated hydrochloric acid (Figure 1) (Tramontini 1973).

3-Amino-substituted 1-phenylpropanols were prepared by reduction of the corresponding aroyl derivatives with $NaBH_4$. We concluded that use of $NaBH_4$ was the method of choice for reduction of the ketones to the corresponding alcohols because yields were higher (Figure 2).

Because the parent compound of these new derivatives had anti-inflammatory and immunomodulating activity (Hadjipavlou-Litina et al 1992), we studied the possible anti-inflammatory and immunomodulating properties of these new compounds. The anti-inflammatory activity of the compounds was evaluated by their ability to inhibit rat paw oedema, the most frequently used test model for anti-inflammatory activity. We used various phlogistic agents, which induce inflammation by the involvement of different mediators, in

Table 5. Effect of the in-vitro addition of compounds **1**–**4** on the mitogenic responses (S.I.) to Con-A of splenocytes from normal and arthritic rats.

Compound **1** ($\mu g\,mL^{-1}$)	Normal S.I.	Arthritic S.I.	Compound **2** ($\mu g\,mL^{-1}$)	Normal S.I.	Arthritic S.I.
0	36	18	0	92	53
1	35	32	1	79	52
2·5	39	26	2	83	49
5	41	27	5	81	31
10	39	16	10	78	1
Compound **3** ($\mu g\,mL^{-1}$)	Normal S.I.	Arthritic S.I.	Compound **4** ($\mu g\,mL^{-1}$)	Normal S.I.	Arthritic S.I.
0	39	19	0	53	37
2·5	40	18	1	47	21
5	36	8	2	41	19
10	30	10	5	34	17
20	25	6	10	23	11

Values are means of triplicate cultures. The results are representative of three similar experiments. S.I. = stimulation index.

Table 6. Production of interleukins 1 and 2 by normal and arthritic rats with or without treatment with compounds **2** and **4**.

Treatment	Interleukin 1 (%)	Interleukin 2 (%)
Normal	100±0·0	100±0·0
Normal + compound **2**	89·1±3·5*	21·6±3·0***
Normal + compound **4**	50·3±4·2**	6·5±0·8***
Arthritic	103·5±5·0	86·9±6·5
Arthritic + compound **2**	150·1±2·3***	48·3±3·0**
Arthritic + compound **4**	67·0±2·7**	6·0±1·0***

The results are representative of three similar experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from result from normal group.

an attempt to elucidate the mode of action of these compounds.

Carrageenan-induced oedema is a non-specific inflammation maintained by the release of histamine, 5-hydroxytryptamine, kinins and later by prostaglandins (Tsurumi et al 1986). The inhibitory effect of acid NSAIDs, such as indomethacin, is usually weak in the first phase (1–2 h), in contrast with their strong inhibition in the second phase (3–4 h) (Gavalas et al 1991). Good inhibition of the second phase of carrageenan-induced oedema was observed for the compounds tested, suggesting they interfere with prostaglandin synthesis. The aroylaminoalcohol derivatives had a stronger inhibitory effect on carrageenan oedema than did the 3-amino-substituted 1-phenylpropanols. We suggest that the carbonyl group is an important structural characteristic of the anti-inflammatory action of these derivatives.

To clarify further the mode of action of these compounds we examined their effects on Con-A-induced oedema. This phlogistic agent induces oedema in which 5-hydroxytryptamine and kinins are involved at the incipient stage (1–2 h). Prostaglandins participate in the late stage of inflammation (3–6 h) (Cottney & Lewis 1975; Masso et al 1994). Under our experimental conditions the anti-inflammatory activity of indomethacin was found to be good in the late stage of Con-A oedema, in which prostaglandins participate, but the compound did not reduce the developing oedema in the first phase of inflammation, as it did for carrageenan-induced oedema. Furthermore, we found that compounds **5** and **6** failed to reduce the oedema induced by Con-A in both the early and late stages, whereas compounds **1**–**4** prevent the development of this inflammation, which is induced by release of kinins and prostaglandins. These results are indicative of different modes of action of aroylaminoalcohols and indomethacin towards this type of oedema and also suggest that Con-A- and carrageenan-induced oedemas are a result, at least to some extent, of different mediators.

Most NSAIDs inhibit the conversion of arachidonic acid to prostaglandins, but have little effect on the metabolism of arachidonic acid to leukotrienes via the lipoxygenase pathway. Yeast and zymosam, which is a structural polysaccharide of the yeast-cell wall, cause an acute inflammatory reaction. Antihistamines, anti-5-hydroxytryptamines and lipoxygenase inhibitors prevent these oedemas in which leukotrienes are also involved, whereas cyclooxygenase inhibitors do not (Nickerson-Nutter & Medvedeff 1996). Other studies suggest that zymosan is a non-specific irritant which elicits not only a leukotriene B_4 response, but also the induction of chemotactic factors C_{3a} and C_{5a} from the activation of the alternative pathway of the complement system, the synthesis of prostaglandins and thromboxanes and the formation and release of platelet-activating factor, free radicals and lysosomal enzymes (Gado & Gigler 1991; Masso et al 1994). Almost all the examined compounds reduced this inflammation considerably, suggesting that the compounds probably interfere with the enzyme lipooxygenase or the complement system, or both, and differ from indomethacin which had a weak inhibitory effect, similarly to all selective inhibitors of the cyclooxygenase system.

Finally, we investigated the inhibitory effects of the compounds on nystatin-induced oedema. Nystatin induces swelling via lysosomal membrane labilization. Other investigators have suggested that prostaglandins also play an important role in this type of oedema (Tsurumi et al 1986). The compounds significantly inhibited nystatin-induced oedema, possibly blocking the action of prostaglandins and stabilizing the cell membranes. In general, the above results indicate that the compounds synthesized affect multiple mediators produced by various cells.

In this study, we have examined the effects of the compounds synthesized, which had good anti-inflammatory activity and different structural characteristics, on immunity-related inflammation, using the adjuvant-induced arthritis model. Adjuvant-induced arthritis is believed to be a consequence of cell-mediated immunity (Panosian et al 1986) to an endogenous virus (Kapusta et al 1979; Vaugham 1990) or to an arthritogenic T lymphocyte clone (Zvaifler 1988; Panayi et al 1992). Thus, we studied the effects of treatment with compounds **2**, **4** and **6** on this model of chronic inflammation. We found that compounds **2** and **4**, in contrast to **6**, delayed the initiation of adjuvant-induced arthritis

in rats and effectively inhibited the secondary lesions. However, these two compounds suppressed the arthritic score in rat joints towards an immunosuppressive activity on the chronic inflammatory response.

The next step was examination of the effects of several of the compounds on the in-vitro mitogenic responses of splenocytes to Con-A, considered to be a T lymphocytes mitogen (Skoog et al 1974), from arthritic and normal rats. It is well established that T lymphocytes are involved in the pathogenesis of adjuvant arthritis. The splenocyte mitogenic responses of arthritic rats to Con-A were reduced, showing that T lymphocytes were affected. It has previously been found that this inhibition of T-cell responses was because of suppressor cells (Kourounakis et al 1979) sensitive to various steroidal and non-steroidal immunosuppressive drugs (Kapusta et al 1979). Treatment with compound **1** augmented the splenocyte mitogenic responses of arthritic rats indicating a possible effect on suppressor cells. The addition of compound **3** to cell culture reduced the mitogenic response of arthritic rats without affecting normal splenocyte responses. These findings indicate that arthritic lymphocytes are sensitive to some mediators possibly produced by arthritic cells activated by compound **3**. In contrast, compound **6** did not have any in-vitro effects on splenocytes from normal and arthritic rats and perhaps for that reason does not have anti-arthritic properties but only moderate anti-inflammatory activity. The mitogenic responses of the adjuvant group with compounds **2** and **4** were reduced in comparison with the normal group, showing that T cells were affected.

Finally, to investigate in more detail the anti-arthritic effects of compounds **2** and **4**, we examined their interference with the in-vitro production of IL-1 and IL-2 from normal and arthritic rats. These two lymphokines are important soluble mediators necessary for optimum in-vitro T-cell proliferation (Duff 1994). It has been postulated that IL-1 and mitogen act synergistically to induce the production of IL-2 and induced IL-2 receptor expression on certain T cells. Thus, IL-2 might interact with certain T cells having IL-2 receptors and cause them to proliferate. Also, these lymphokines participate in the development of human rheumatoid and adjuvant arthritis (Oppenheim et al 1982; Duff 1994). We found that when compound **2** was added to cultures of macrophages from arthritic rats the amounts of IL-1 produced were greater than by the control group. These findings suggest that compound **2** resembles the classical cycloxygenase inhibitors, which increase the production of IL-1 (Dinarello et al 1984; Lim & Stewart 1990). In contrast, the presence of compound **4** suppressed the production of IL-1 from normal and arthritic rats, indicating a possible inhibitory effect on macrophages; this differentiates compound **4** from compound **2**. The amount of IL-2 produced from normal and arthritic-stimulated T cells, and the mitogenic responses, were reduced by both compounds. These data indicate that their anti-arthritic action possibly is a result of the suppression of the production or the activity of IL-2.

In conclusion, the series of aroylaminoalcohol derivatives were more potent anti-inflammatory agents than the 3-amino-substituted 1-phenylpropanols. In an attempt to correlate structural features and physicochemical characteristics with biological properties, we can only state, because of the small number of compounds examined, the possiblity of trends. The presence of the aroyl group seems to give the molecules more activity than those having this group reduced to the corresponding alcohol; the more lipophilic the compound the greater the biological activity. The findings of this work suggest that the compounds tested prevent the release of many of the chemical mediators that participate in the inflammatory process, or limit their biological action, or both. In addition compounds **2** and **4** had anti-arthritic effects on adjuvant-induced arthritis probably because of their immunomodulatory properties on the immune system. Therefore, for the prepared structures, it is the combination of the anti-inflammatory and immuno-modulating properties that might contribute to the control of both the acute and the chronic inflammation.

Acknowledgements

The authors wish to thank the General Greek Ministry of Research and Technology for partial financial support.

References

Calhoun, W., Carlson, R. P., Crossley, R., Datko, L. J., Dietrich, S., Heatherington, K., Marshall, L. A., Meade, P. J., Opalko, A., Shepherd, R. G. (1995) Synthesis and anti-inflammatory activity of certain 5,6,7,8-tetrahydroquinolines and related compounds. J. Med. Chem. 38: 1473–1481

Cottney, J., Lewis, A. J. (1975) Mechanisms of concanavalin A-induced inflammation in the rat. Br. J. Pharmacol. 53: 445P

Dinarello, C. A., Bisham, I., Roisenvasser, I. J., Coceani, F. (1984) The influence of lipoxygenase inhibitors on the in-vitro production of human leucocytic pyrogen and lymphocyte activating factor (IL-1). Int. J. Immunopharmacol. 6: 43–50

Duff, G. W. (1994) Cytokine and acute phase proteins in rheumatoid arthritis. Scand. J. Rheumatol. 23 (Suppl. 100): 9–19

Fawcett, P. T., Dubbs, S. B., Fawcett, L. B., Doughty, R. A. (1990) Induction of humoral manifestations of autoimmunity following intraperitoneal injection of complete Freund's adjuvant in mice. Autoimmunity 6: 249–256

Gado, K., Gigler, G. (1991) Zymosan inflammation a new method suitable for evaluating new antiinflammatory drugs. Agents Actions 32: 119–121

Gavalas, A., Kourounakis, L., Litina, D., Kourounakis, P. (1991) Anti-inflammatory and immuno-modulating effects of the novel agent γ-(2-aminoethylamino)-2-butyrothienone: inhibitory effects on mouse paw oedema. Arzneim. Forsch. 41: 423–426

Hadjipavlou-Litina, D., Rekka, E., Hadjipetrou-Kourounakis, L., Kourounakis, P. N. (1992) Ionization constants, lipophilicity and biological studies of some novel aryl-(amino or hydroxy) ethylamino ketones with anti-inflammatory activity. Eur. J. Med. Chem. 27: 1–6

Kalsi, R., Rande, K., Bhalla, T. N., Barthwal, J. P., Gupta, G. P., Parmar, S. S. (1990) Anti-inflammatory activity of quinazolinoformazans. J. Pharm. Sci. 79: 317–320

Kapusta, M. A., Young-Rodenchuk, M., Kourounakis, L. (1979) Restoration of diminished splenic responses to phytohemaglutinin and concanavalin A in adjuvant induced disease by virazole: possible pathogenetic role of virus and suppressor cells. J. Rheumatol. 6: 507–518

Kourounakis, L., Kapusta, M. A. (1974) Effect of Freund's adjuvant on the mitogenic responses of rat lymphocytes. Ann. Rheum. Dis. 33: 185–189

Kourounakis, L., Kapusta, M. (1976) Restoration of diminished T-cell function in adjuvant induced disease by methotrexate: evidence for two populations of splenic T-cell suppressors. J. Rheumatol. 3: 346–354

Kourounakis, L., Kapusta, M. A., Young-Rodenchuk, M. (1979) Restoration of diminished splenic responses to phytohemaglutinin and concanavalin A in adjuvant induced disease by corticosteroids: further evidence for the presence and pathogenetic role for two populations of splenic suppressor cells. J. Rheumatol. 6: 497–506

Kourounakis, L., Gavalas, A., Karagouni, E. E., Litina, D., Kourounakis, P. (1991) Anti-inflammatory and immunomodulating effects of the novel agent γ-(2-aminoethylamino)-2-butyrothienone: inhibitory effect on rat adjuvant induced disease. Arzneim. Forsch. 41: 423–426

Lim, W. H., Stewart, A. G. (1990) Macrophage activation reduces mobilization of arachidonic acid by guinea-pig and rat peritoneal macrophages in-vitro. Agents Actions 31: 290–297

Lipsky, P. E., Davis, S. L., Cush, J. J., Oppenheimer-Marks, N. (1989) The role of cytokines in the pathogenesis of rheumatoid arthritis. Springer Semin. Immunopathol. 11: 123–162

Masso, J. M., Villar, A. M., Conde, J. R., Martorell, J. (1994) Mechanism of anti-inflammatory action of fepradinol. Arzneim. Forsch. 44 : 68–74

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immun. Methods 65: 55–63

Nickerson-Nutter, C. L., Medvedeff, E. D. (1996) The effect of leucotriene synthesis inhibitors in models of acute and chronic inflammation. Arthritis Rheum. 39: 515–521

Oppenheim, J. J., Stadler, B. M., Siraganian, R. P., Mage, M., Mathieson, B. (1982) Lymphokines, their role in lymphocyte responses: properties of interleukin 1. Fed. Proc. 41: 257–262

Panayi, G. S., Lanchbury, I. S., Kingsley, G. H. (1992) The importance of the T-cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis Rheum. 35: 729–735

Panosian, N., Heyner, S., Copetola, R. A., Oryechowski, R. F. (1986) Humoral and cellular immunologic aspects of adjuvant and collagen arthritis in rats. J. Immumopharmacol. 8: 347–370

Skoog, V. T., Weber, T. H., Richter, W. (1974) Studies on the interaction between mitogens and human lymphocyte in vitro. Exp. Cell Res. 85: 339–350

Tramontini, M. (1973) Advances in the chemistry of Mannich base. Synthesis 703–775

Tsurumi, K., Kyuki, K., Niwa, M., Kokuba, S., Fujimura, H. (1986) Pharmacological investigations of the new antiinflammatory agent 2-(10,11-dihydro-10-oxodibenzo[*b,f*]thiepin-2-yl)propionic acid. Arzneim. Forsch. 36: 1796–1803

Vaugham, J. H. (1990) Infection and rheumatic disease. Bull. Rheum. Dis. 39: 1–8

Yiangou, M., Hadjipetrou-Kourounakis, L. (1989) Interleukins production and responsiveness in Fischer rats with adjuvant-induced disease: role of suppressor cells. J. Clin. Lab. Immunol. 28: 85–90

Zvaifler, N. J. (1988) New perspectives on the pathogenesis of rheumatoid arthritis. Am. J. Med. 85: 12–17