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The Study of the Effect of Histidine Derivatives as a Novel Antinociceptive and Anti-Inflammatory Activity

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Abstract: This study explains the biochemical activity of histidine derivatives The compounds were identified by CHN analysis, FT-IR and H^1 NMR. The results certified that the chemical structures of the prepared compounds. The anti-inflammatory and antinociceptive activity was studied by two different tests; the hot plate test and writhing test for analgesic activity and two tests for anti-inflammatory activity they are formalin induced inflammation test and carrageen an induced inflammation test. The histidine derivatives were found to have potent activity as anti-inflammatory and antinociceptive. The active compounds were tested to acute toxicity. It was found that they are safety to the dose 5 g/kg orally in mice without any mortality.

Keywords: Histidine derivative, Anti-inflammatory agent, Antinociceptive agent

Introduction

Aspirin was the first drug developed as a marketable drug in 1897 but other salicylates have long been used in the treatment of pain. The ancient Greeks, for example, used the bark of willow trees for pain relief. Aspirin blocks the production of some eicosanoids and thus relives the symptoms of pain and reduces fever. The active ingredient of aspirin, acetylsalicylic acid, irreversibly inhibits COX activity by transferring an acetyl group to an active site serine residue of the bifunctional enzyme. By blocking the activity of COX, aspirin prevents the formation of a variety of eicosanoids that were synthesized after the COX reaction[1,2].

Experimental

The amino acid *L*-histidine (1.55 g, 0.01 mol) was dissolved in ethanol (10 mL) and a solution of potassium hydroxide (0.56 g, 0.01 mol) in ethanol (10 mL) was added, the reaction mixture were refluxed for 2 h, the solution were evaporated by rotary evaporator. The residue was recrystallized from methanol, A white precipitate (compound 1) *L*-histidine ethyl ester was formed (ethyl 2-amino-3-(1*H*-imidazole-4-yl)propanoate). M.p.198 °C, yield 72.1%.

Acetyl histidine derivatives[4,5]

L-Histidine (1.55 g, 0.01 mol) was dissolved in (15 mL) glacial acetic acid and was treated with (1 mL, 0.01 mol) acetic anhydride. The mixture was boiled for 2 h. The solution was cooled to room temperature and evaporated in rotary evaporator to dryness. The residue was dissolved in 20 mL of water and extracted twice in a separating funnel with 15 mL carbon tetrachloride. The aqueous layer was evaporated and the residue was taken up twice in about 20 mL of distilled water and the solution each time was evaporated in rotary evaporator to dryness. The residue was dissolved in a few mL of distilled water then filtered. To the filtrate 20 mL of acetone was added, the mixture was cooled to 5 ºC for several hours. The precipitate were filtered under vacuum and washed with 70% acetone and then with 100% acetone and finally with dry ether. The precipitate recrystallized with ethanol to afford *N*α-acetyl-*L*-histidine; 4-Imidazole-4yl-2-(*N*-acetyl)propanoic acid (Compound 2). M.p.153 °C, yield 68.9%.

Compound 3 was prepared by reflux (1.83 g, 0.01 mol) of compound 1 and 10 mL glacial acetic acid for 2 h. The solution were cooled to room temperature and evaporated in rotary evaporator to dryness. The residue was dissolved in 20 mL distilled water and extracted twice in separating funnel with 15 mL of carbon tetrachloride. The aqueous layer was evaporated and the residue was taken up in about 20 mL of water and the solution were evaporated in rotary evaporator to dryness. The residue was dissolved in a few mL of distilled water then filtered. To the filtrate 20 mL of acetone was added and the mixture was cooled to 5 ºC for several hours. The precipitate were filtered under vacuum and washed with 70% acetone and then with 100% acetone, and finally with dry ether. The precipitate recrystallized with ethanol to afford *N*α-acetyl-*L*-histidine ethyl ester; Ethyl-2-acetamido-3-(1*H*-imidazole-4-yl) propanoate (compound 3)[6]. M.p. 118 °C, yield 63.4%.

Propanoyl histidine derivative[7]

Dried *L*-histidine (1.55 g, 0.01 mol) was dissolved in 10 mL dried propanoic acid. The mixture was refluxed for 2 h. The propanoic acid was evaporated in rotary evaporator to dryness. The residue was dissolved in 20 mL of distilled water and extracted twice in separating funnel with 15 mL of chloroform. The aqueous layer was evaporated and the residue was taken up twice in about 20 mL of distilled water and the solution each time was evaporated in rotary evaporator to dryness. The residue was dissolved in few mL of distilled water and filtered. To the filtrate 20 mL of acetone was added and the mixture cooled to 5 °C for several hours. The precipitate were filtered under vacuum and washed with 70% acetone and then with 100% acetone and finally with dry ether. The precipitate recrystallized with ethanol to afford *N*α-propanoyl-*L*-histidine; 3-(1*H*-imidazole-4-yl)-2-propanamido propanoic acid (compound 4). M.p. 191-192 °C, yield 61.6%.

In the same way the compound 4 was prepared by refluxed compound 1 (1.83 g, 0.01 mol) with 10 mL of dried propanoic acid to give *N*α-propanoyl-*L*-histidine ethyl ester; ethyl-3-(1*H*-imidazole-4-yl)-propanamido propanoate (Compound 5). M.p. 234-236 °C, yield 43.16%.

Pharmacology

Animals

Male and female albino mice (20-30 g) were obtained from the animal house of university of Basrah, college of sciences. Animals were housed in colony room (12/12 h) light/ dark cycle at 23±2 ºC and had free access to food and water. Mice were divided into six groups in each experiment (n=6, 3 male and 3 female).

Drugs preparation

The drugs used in the experiments were diluted in a way to obtained injection volume of 0.2 mL. Each drug was dissolved in appropriate solvent as following: aspirin and diclofenac in water; carrageenan 1% in normal saline; acetic acid 0.7% in water; formalin 1% in water.

Antinociceptive activity[8]

Hot plate test

A mouse was placed on a hot plate maintained at 56±2 ºC and the latency of its reaction to this nociceptive stimulus (number of seconds before it licked its paw or jumping) was quantified, with interruption time of 20 sec. The latency was measured just before zero time and 1, 2, 3 and 4 h after injections. The hot plate test was carried out to assess the effects of agent on the thermal nociceptive threshold. The compounds were given orally (50 mg/kg) and control group received (0.2 mL) distilled water orally. Aspirin (50 mg/kg) and diclofenac (25 mg/kg) were used as test standards.

Writhing test[9]

The antinociceptive effect was evaluated by the writhing test, induced by acetic acid (0.7%) v/v (0.2 mL) intrapretonial injected to mice. Then the mice placed in large glass beaker and intensity of nociception was quantified by counting the total numbers of writhing occurring between zero and 30 min after stimulus injection. The writhing response consists of a contraction of the abdominal muscles together with a stretching of the hind limbs. The compounds 50 mg/kg orally administered 1 h before the nociceptive agent in treated group. Five minutes after the acetic acid injection we observed the numbers of writhes for a period of 25 min. Control group was received (0.2 mL) distilled water orally. Aspirin (50 mg/kg) and diclofenac (25 mg/kg) were used as test standards.

Anti-Inflammatory activity

Formalin induced inflammation test[10,11]

The inflammation was produced by subaponeurotic injection of 20 µL of 1% formaldehyde in the left hand paw of the mice on the first hour after the oral administration of the compounds 50 mg/kg orally. The control group was received distilled water (0.2 mL). Aspirin (50 mg/kg) and diclofenac (25 mg/kg) were used as references drugs. The changes in paw size was measured at zero-hour and then at 1 h intervals up to the 5 h with a micrometer device.

Carrageenan induced inflammation test [12]

For the determination of the effects on acute inflammation, carrageenan induced inflammation model was used. Inflammation was induced by sub-planter injection of a homogenous suspension of 1% carrageenan in physiological saline 25 µL. Mice were orally given compounds in a single dose 50 mg/kg orally one hour before the induction of paw inflammation. The control group was given aspirin (50 mg/kg) and diclofenac (25 mg/kg) orally one hour before the induction, while the blank group was received distilled water (0.2 mL) only. Paw size was measured in every mouse hourly during four hours after the induction with a micrometer device[12].

Statistical analysis[13,14]

All values in the tables and the figures are expressed as the mean ± standard error. The comparisons between groups were made by the analysis of variant student's *t*-test (2-tailed, 3-typed) two-tailed distribution, two-sample unequal variance, with microsoft excel software. Differences with $P<0.05$ between experimental groups were considered statistically significant.

Acute toxicity[15]

In order to verify the LD_{50}, mice (n=10; 5 male and 5 female) in each group administered orally following single dose of (1, 3 and 5 gm/kg) to each compound dissolved in (0.5 mL) distilled water, control group received 0.5 mL distilled water only. The mortality was observed during 24 h.

Result and Discussion[16,17]

Thin layer chromatography (TLC) of starting materials and products were preformed using suitable solvents. The spots visualized by using ultraviolet light lamp at 254 nm instrument, iodine vapour, 10% sulphuric acid and ninhydrin.

The mobile phase of TLC was propanol: water (1:1). Compounds (1, 3 and 5) give positive to ester test and compounds (2-5) give positive to secondary amine test with two test benzene sulfonyl chloride and diazonium ion. The esterification of carboxylic acids with alcohols is a reversible reaction and it can be accomplished only if a means is available to drive the equilibrium to the right. There are many ways of doing this and in this case by adding excess of alcohol to driving the equilibrium to the products. Table 1 shows the physical properties of the prepared compounds while Table 2 indicates the CHN chemical analysis of compounds 3 and 5. Elemental analyses were performed for the final compounds to confirm their basic chemical compositions. The measured percentages show a reasonable good agreement with calculated results.

Table 3 shows the major bands of the histidine derivatives. The NH of imidazole and α-NH bond stretching was between (3444-3282 cm^{-1}), methylene hydrogen bond C=CH between (3168-3016 cm^{-1}) and the aliphatic hydrogen attributed between (2980-2820 cm^{-1}). The carbonyl bond was absorbed between (1714-1587 cm^{-1}), the C=C bond was stretching between (1400-1433 cm^{-1}) and the NH bending between (1587-1535 cm^{-1})[18]. Figures 1 to 4 show the FT-IR spectrums of compounds.

Table 1. Characterizations and physical data of histidine derivatives

Comp	Compound name	M.wt	m.p.^{o}C	Colour	R_f	%Yield
1	*L*-histidine ethyl ester	183.2	198	White crystals	0.61	72.1
2	*Nα*-acetyl-*L*-histidine	197.2	153	White powder	0.67	68.9
3	*Nα*-acetyl-*L*-histidine ethyl ester	225.3	118	White powder	0.65	63.4
4	*Nα*-propanoyl-*L*-histidine	211.2	191-192	Pale yellow powder	0.53	61.6
5	*Nα*-propanoyl-*L*-histidine ethyl ester	239.3	234-236	Beige powder	0.49	43.16

Table 2. Elemental analyses of compounds

Compound	Molecular formula	M.wt	Elemental analysis %			
				C	H	N
3	$C_{10}H_{15}N_3O_3$	225.3	Cal.	53.3	6.65	18.64
			Found.	52.74	6.63	18.28
5	$C_{11}H_{17}N_3O_3$	239.3	Cal.	55.16	7.1	17.55
			Found.	55.95	7.38	17.72

Table 3. Characteristic FT-IR absorptions (cm^{-1}) of histidine derivatives

Comp.	Stretching υ_{N-H}	Stretching $\upsilon_{C=C-H}$	Stretching υ_{C-H}	Stretching $\upsilon_{C=O}$	Stretching $\upsilon_{C=C}$	Bending δ_{N-H}
1	3444, 3419	3091, 3016	2820	1640	1410	1587
2	3282,	3140, 3020	2935, 2870	1714, 1654	1400	1540
3	3413	3147, 3031	2893	1716, 1639	1433	1542
4	3338	3099, 3016	2980, 2863	1633	1403	1535
5	3338	3168, 3109	2975, 2940, 2860	1641, 1587	1404	1535

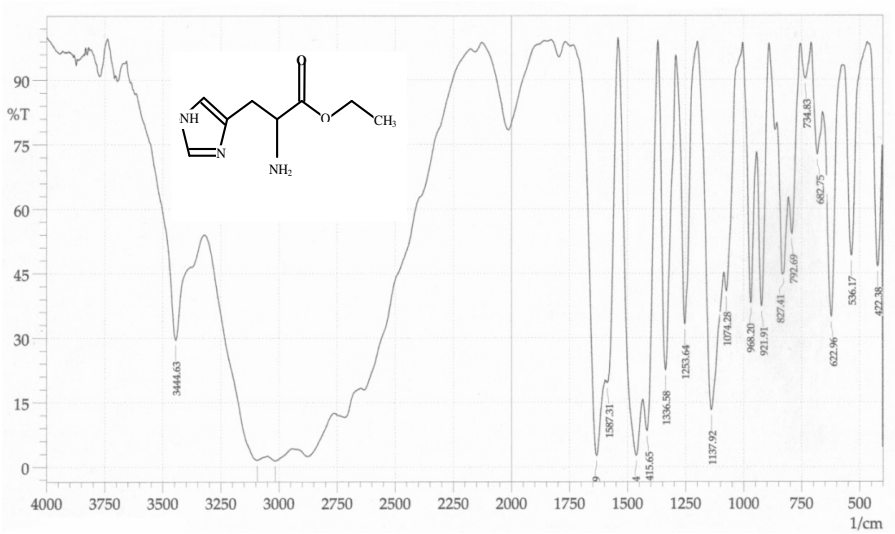

Figure 1. The FT-IR spectrum of compound 1

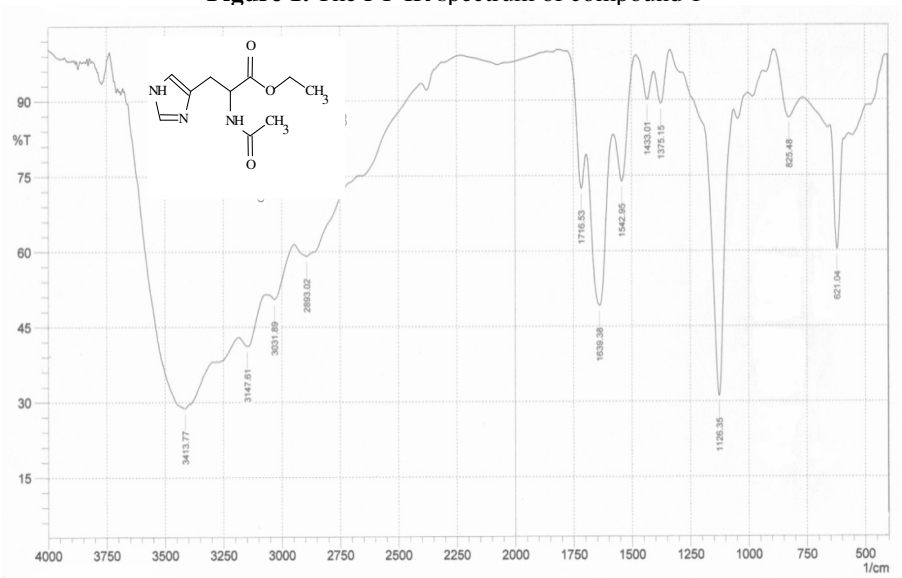

Figure 2. The FT-IR spectrum of compound 3

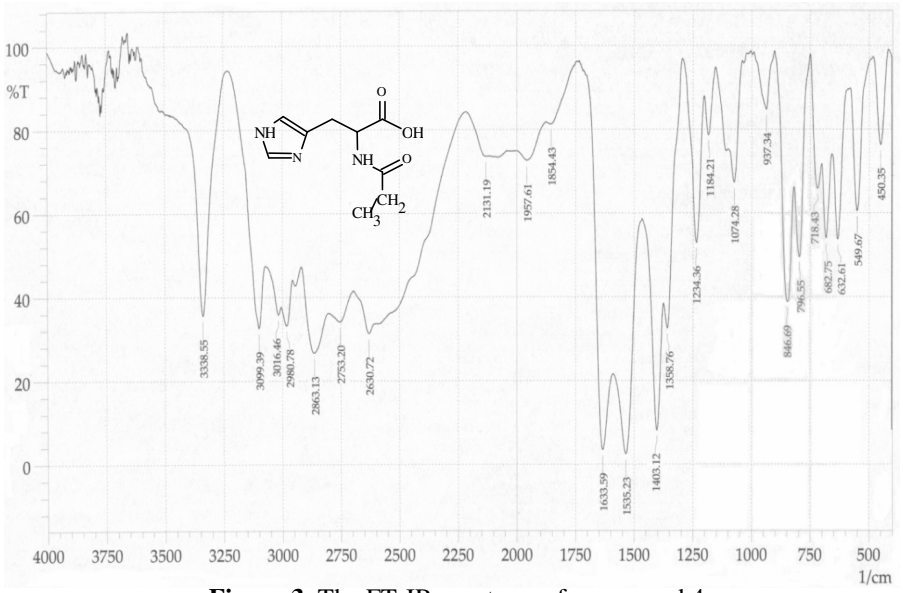

Figure 3. The FT-IR spectrum of compound 4

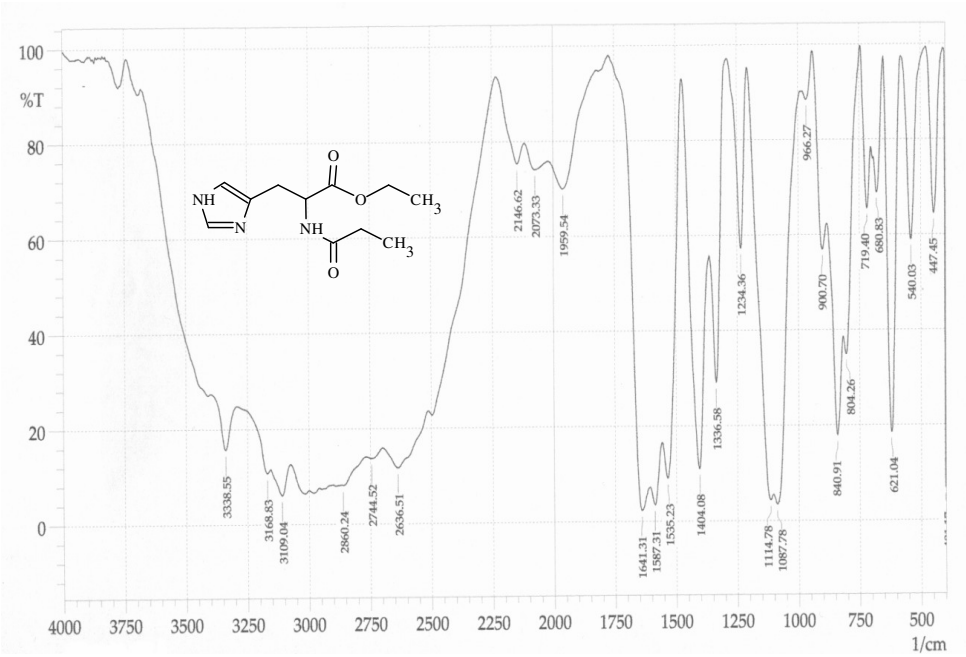

Figure 4. The FT-IR spectrum of compound 5

The ^{1}H NMR spectra of compounds 1 and 4 shows the NH peak at 7.7 ppm and 8.02 ppm respectively, the hydrogen in imidazole ring peak between 8.33-6.8 ppm, CH 4.03 and 4.39 ppm respectively, CH_2 between 3.19- 2.8 ppm and CH_3 1.1 and 0.97 ppm respectively. The Table 4 and the Figure 5 and 6 show the characteristics and NMR spectra of compounds 1 and 4.

Table 4. Characteristics H^1 NMR of compounds 1 and 4

Comp	δ NH	imidazole		δ CH-CH_2	δ CH_2-CH_3
		δ N=C<u>H</u>-NH	δ C=C<u>H</u>-NH		
1	7.7 s	8.33 s	7.22 s	4.03 t CH, 3.19,3.03 m CH_2	3.74 d CH_2, 1.1 t CH_3
4	8.02 d	7.63 s	6.8 s	4.39 q CH, 2.92, 2.8 m CH_2	2.11 q CH_2, 0.97 t CH_3

s = Singlet, d = doublet, t = triplet, q = quartet, m = multi

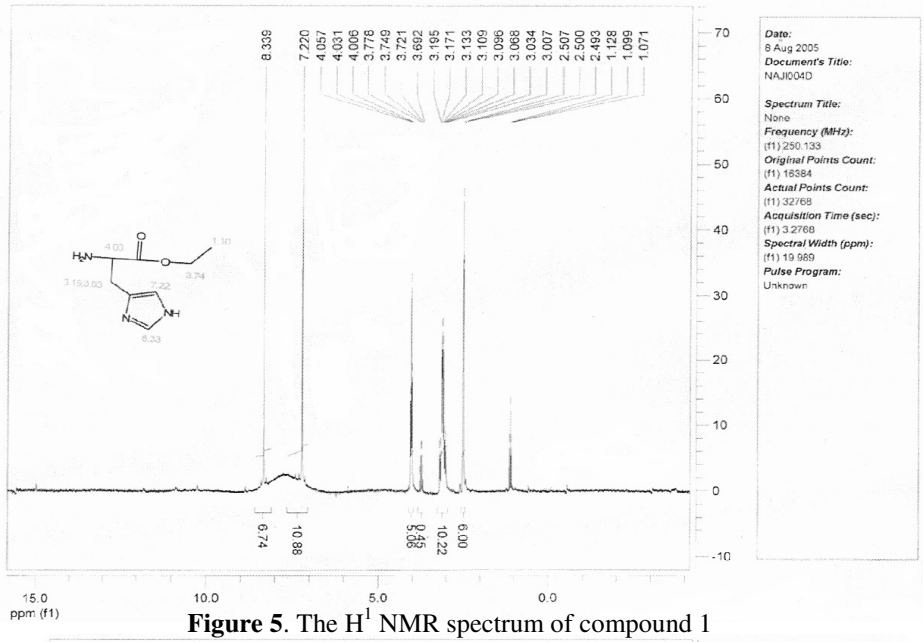

Figure 5. The H^1 NMR spectrum of compound 1

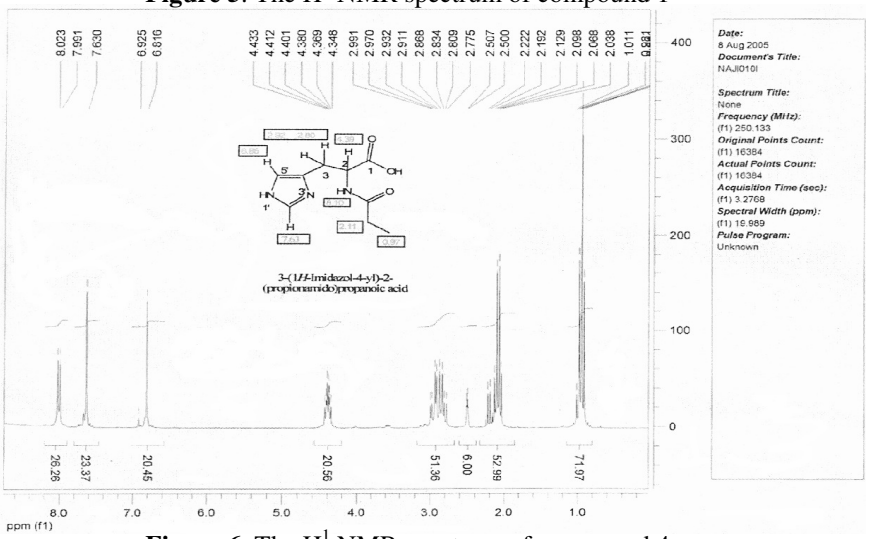

Figure 6. The H^1 NMR spectrum of compound 4

Table 5 and Figure 7 shows the results of hot plate test. All derivatives were found to have analgesic effect in hot plate test, compound 3 (*Nα*-acetyl-*L*-histidine ethyl ester) was more effective than other histidine derivatives. The substitution on both amino and carboxyl groups of histidine increases the activity of drug. Compound 5 (*Nα*-propanoyl-*L*-histidine ethyl ester) was less effective than compound 3 but compound 4 (*Nα*-propanoyl-*L*-histidine) still more effective than the other derivatives thus the substitution on amino group must not be more than three carbon groups (C_3) and it was found that two carbon groups (C_2) are more effective than three carbon groups if carboxyl group was substituted. This may be due to the size of the small molecule to be sitting in an active site of enzyme[19].

Table 5. Hot plate tests of histidine derivatives

Comp.	Latency time in sec				
	0 h	1 h	2 h	3 h	4 h
Control	3.4±1.37	3.7±1.5	4.4±1.75	5.6±2.5	4±0.5
Aspirin	2±1.0	4.8±1.25	9±1.5 *	7.2±2.75	9.11±1.0 *
Diclofenac	3±0.5	7.8±3.25	14±2.5 *	14.1±3.5 *	9.6±3.5
1	4.6±2.11	12.6±2.8 *	8.33±1.1	15.5±4.1 *	24±4.2 2*
2	6±2.4	13.4±3.3 *	17.6±1.5 *	16±1.7 *	20.4±2.2 2*
3	5.2±2	17.5±2.5 *	31±3.1 3*	28.2±2.8 3*	28±3.3 3*
4	4.5±2.2	17.2±1.6 *	18.4±2.3 2*	18.6±2.4 2*	13.5±1.3 *
5	5.33±1.1	7.66±1.55	7.66±2.88	22.3±2.78 2*	18.3±2.22 2*

* *=P<0.05; 2* =P<0.01; 3* =P<0.005*

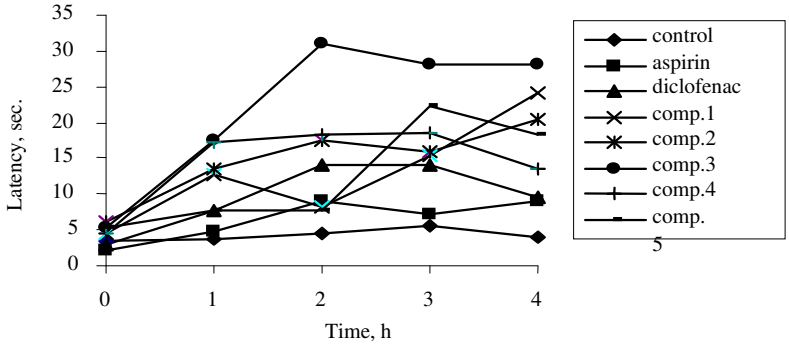

Figure 7. Hot plate tests of histidine derivatives

Table 6 and Figure 8 shows the result of writhing test of histidine derivatives. Compound 1 (*L*-histidine ethyl ester) and compound 4 (*Nα*-propanoyl-*L*-histidine) show significant effect (comp1 $p<0.05$, comp4 $p<0.05$). The activity increased when carboxyl group was substituted, but decreased when amino group was substituted except compound 4 which is substituted with three carbons (C_3) group and carboxyl group was free.

Table 6. Writhing tests of histidine derivatives

control	aspirin	diclofenac	comp.1	comp.2	comp.3	comp.4	comp.5
43±2	6.3±2 5*	10.6±2 5*	27±3 *	36±2.8	42±3	28±2 *	44±2.5

* *=P<0.05; 2* =P<0.01; 3* =P<0.005; 4* = P<0.001; 5* =P<0.0005*

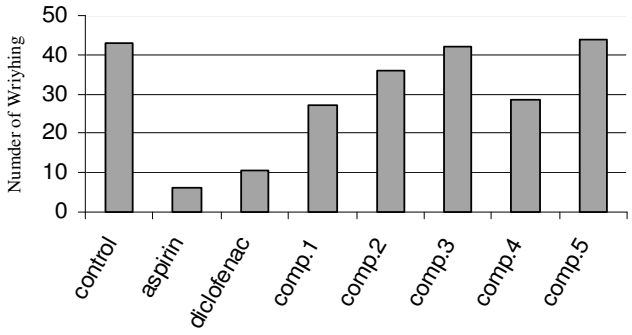

Figure 8. Writhing tests of histidine derivatives

Table 7 and Figure 9 shows the result of formalin induced inflammation test. The substitution on both carboxyl and amino groups of histidine increased anti-inflammatory activity and substitution on carboxyl group (Compound 1) was more effective than substituted on amino group (Compound 2 and 4).

Table 7. Formalin induced inflammation tests of histidine derivatives

comp.	change of paw size, mm×10 $^{-2}$					
	0 h	1 h	2 h	3 h	4 h	5 h
control	0±2	77±2.5	96±9	84±2	91±14	87±14
aspirin	0±10	52±16	77±21	61±13	40±12 *	38±11.5*
diclofenac	0±23	33.5±21^{2*}	52.5±25	57.5±25	22.5±20^{2*}	43±24
1	0±9	43±10 *	48±22	31±16^{2*}	29±9^{2*}	25±21^{2*}
2	0±15	64±15	65±11	59.5±13	61±15	72±23
3	0±17	37±18^{2*}	25±15^{3*}	30±14^{2*}	3±8^{4*}	1±3^{5*}
4	0±13	54±21	62±14	71±18	32±16^{2*}	46±19
5	0±8	32±14*	17±7 ^{2*}	20±6 ^{3*}	21±6.2^{2*}	18±4.4 ^{3*}

* *=P<0.05;* 2^{*} *=P<0.01;* 3^{*} *=P<0.005;* 4^{*} *= P<0.001;* 5^{*} *=P<0.0005*

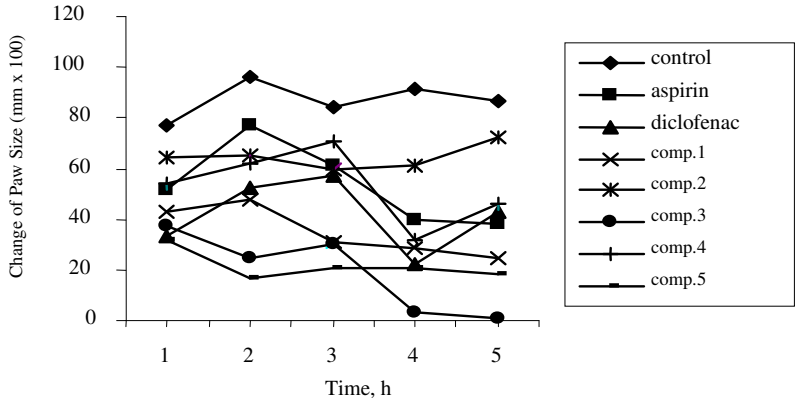

Figure 9. Formalin induced inflammation tests of histidine derivatives

Table 8 and Figure 10 shows the result of carrageenan induced inflammation test of histidine derivatives. All derivatives were given significant effects. Compound 3 was more effective than the other compounds because it is substituted on positions amine and carboxyl group, then compound 2 because it substituted on amino group.

Table 8. Carrageenan induced inflammation tests of histidine derivatives.

Comp.	change of paw size, mm×10^{-2}					
	0 h	1 h	2 h	3 h	4 h	5 h
Control	0±5	140±15	111±21	112.5±12	120±15	95±25
Aspirin	0±22	118±1	90±2	64±4	80±2	77±5
Diclofenac	0±1	85±15	85±29	58.5±3	72±22	62±7
1	0±12	82±20	60±25	69±10	59±9	75±20
2	0±7	52±23	43±21	32.5±15*	34±17*	29±12*
3	0±16	82±18	19±6^{3*}	29±14*	39±8*	36±5*
4	0±19	52±16	41±12	36±16*	51±6	50±18
5	0±11	90±24	59±17	58±13	53.5±14	60±12

** =P<0.05; 2* =P<0.01; 3* =P<0.005*

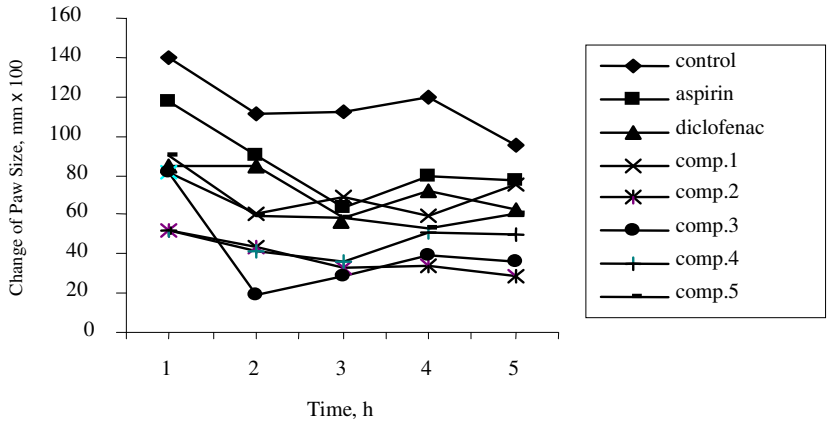

Figure 10. Carrageenan induced inflammation test of histidine derivatives

The injection of carrageenan increased in leukocyte infiltration and accumulation of PGE2, LTB4 and nitrate. Carrageenan also induced elevated expression of cyclooxygenase-1 and -2, inducible nitric oxide synthase and 5-lipoxygenase in the inflammatory exudates[20].

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