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Synthesis and Anti-inflammatory and Analgesic Activities of Phenoxyalkanoic Acid Derivatives

Kuk Hyun Shin[1], Eun Bang Lee[1], Sang Woo Park, Kye Jung Shin, Dong Chan Kim and Dong Jin Kim

Division of Applied Sciences, Korea Institute of Science and Technology, Seoul 136-791 and [1]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

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The synthesis of phenoxyalkanoic acid derivatives and their anti-inflammatory and analgesic activities are described. Analysis of structure-activity relationships shows that in trichlorophenoxy derivatives anti-edematous potency is associated with the presence of 1-thiopropyl moiety and 2 or 4-aminopyridyl moiety at R' position contributes to the analgesic activity.

Key words : Phenoxyalkanoic acid derivatives, Anti-inflammatory activity, Analgesic activity, Carrageenin-induced paw edema, Acetic acid writhing syndrome, Tail clip pressure test

INTRODUCTION

Since the development of salicylates as anti-inflammatory drugs at the end of the nineteenth century (Hanzlik, 1927), glucocorticoids have been demonstrated to be highly effective on both acute and chronic inflammatory responses (Hench *et al.*, 1949). However, due to their severe untoward side effects such as osteoporosis, ulcerogenicity, salt retention, etc. (Goodman and Gilman, 1975), efforts to find out clinically potent non-steroidal anti-inflammatory substances without side effects have been pursued, and as a result, indomethacin, phenylbutazone(Domenjoz, 1966) and other aryl acetic acid derivatives became available (Mizushima, 1968). Although several arylalkanoic acids as strong non-steroidal anti-inflammatory agents are currently used not only clinically but also experimentally and cause fewer gastrointestinal distress than does aspirin, some untoward side effects such as gastric ulcer, headaches and other central effects especially in long term therapy still remains as significant clinical problems.

The present study, therefore, aims at pursuing new type anti-inflammatory and analgesic compounds with arylalkanoic acid moiety which devoid of such untoward side effects. In this report, we describe the synthesis, analgesic and anti-inflammatory screening data and structure-activity relationships for a series of aryloxyalkanoic acid derivatives. Phenols reacted readily in water with chloroalkanoic acids from which several derivatives of amides and esters were synthesized and their activities were tested. Among these compounds tested only a few were of some importance for further investigations.

Correspondence to: Kuk Hyun Shin, Natural Products Research Institute, Seoul National University, 28, Yeonkeun Dong, Chongro-Ku, Seoul 110-460, Korea

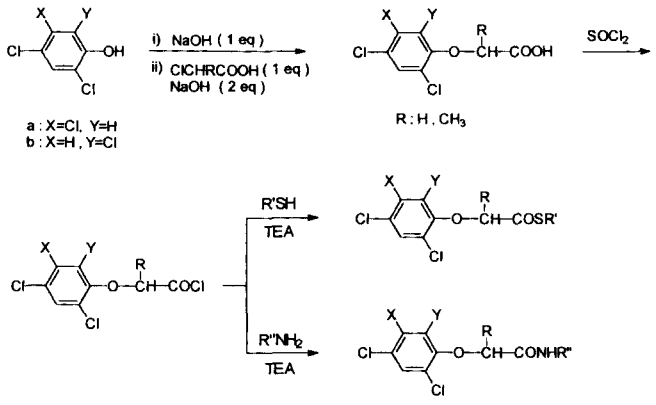
MATERIALS AND METHODS

Instruments and chemicals

^{1}H-NMR spectra were measured at 60 MHz on JEOL and chemical shifts were reported in δ units relative to internal tetramethylsilane. Melting points were taken on a Thomas Hoover capillary melting point apparatus and are uncorrected. TLC was performed on precoated silica gel (0.25 mm, 60F 254, Merck) and column chromatography on Kiesel gel, 70-230 mesh, Merck. Solvents such as THF, methylene chloride, benzene and alcohol were purified by distillation from sodium benzophenone ketyl, P_2O_5, metal sodium and magnesium alkoxide, respectively. Other chemicals and reagents are first grades commercially available.

General procedure for the synthesis of phenoxy alkanoic acid derivatives

Phenoxyalkanoic acid derivatives were prepared with various phenols which were commercially available or prepared from 2-chloro-4-methylphenol by chlorination with SO_2Cl_2. Treatment of phenols with NaOH and chloroacetic acid or 2-chloropropionic acid in toluene at 110°C gave phenoxyacetic acid, respectively. Upon heating with excess thionylchloride, phenoxyalkanoic acids underwent smoothly



Scheme 1.

to acid chlorides, which were purified by vacuum distillation. Finally, treatment of acid chlorides with thiols and amines in the presence of triethylamine as a base afforded esters, thioesters and amides in good yields, respectively (Scheme 1).

Pharmacological methods

Animals and treatments: Male Sprague-Dawley rats weighing 200-250 g and ICR mice weighing 20-25 g were used. The test compounds suspended in 0.5% CMC were administered orally. Aminopyrine and hydrocortisone were used for comparison as positive reference drugs.

Carrageenin induced paw edema test: Carrageenin edema assay was carried out according to the method of Winter *et al.* (1962). Groups of 10 rats were administered with the indicated doses of test compounds 30 min prior to the injection of carrageenin (0.1 ml of 1%, intradermally) as an irritant. The volume of edema was measured both before and 1 hr intervals from 1 hr upto 5 hr after the injection of the irritant.

Analgesic activity test: Analgesic activity was evaluated by acetic acid-induced writhing syndrome assay and tail clip test in mice according to the method of Whittle (1964) and Takagi *et al.* (1966), respectively. In acetic acid writhing test, groups of 10 mice were administered with the indicated doses of test compounds 1 hr prior to the injection of acetic acid (0.2 ml/20 g b.w. of 0.7%, *i.p.*). The numbers of writhing syndrome were counted 10 min later for 10 min and the percent inhibition of writhing relative to the control was calculated. In tail clip test, groups of 10 mice were administered with the indicated doses of the test compounds 1 hr prior to the induction of 500 g pressure with aortic clip and the numbers of mice responded to avoid damage within 6 seconds were counted.

Acute toxicity test: Test compounds were administered and the number of mice died within 72 hr were observed to calculate MLD values.

RESULTS AND DISCUSSION

Table I shows physicochemical and spectral data on 25 phenoxy alkanoic acid derivatives which were readily synthesized in good yield using the standard synthetic procedures as described in the experimental section. The compounds synthesized were purified on silica gel and the structures were identified by ^{1}H-NMR data as indicated in Table I.

The compounds thus synthesized were subjected to

Table I. Chemical data on synthetic compounds

Comp.	H	Xn	R	R'	mp(°C)[a]	NMR data
01	CH	2,4,5-Cl	H	4-aminopyridyl	214-216	4.9(s, 2H), 7.2-8.3 (m, 6H), 10.6(s, 1H)
02	CH	2,4,5-Cl	H	3-aminopyridyl	214-216	4.9(s, 2H), 7.2-8.7 (m, 6H), 10.4(s, 1H)
03	CH	2,4,5-Cl	H	4-fluoroaminophenyl	189-191	4.9(s, 2H), 7.0-7.9 (m, 7H)
04	CH	2,4,5-Cl	H	2,4-difluoroaminophenyl	183-185	5.0(s, 2H), 7.2-8.0 (m, 5H), 9.8(s, 1H)
05	CH	2,4,5-Cl	H	1-thiopropyl	64-66	1.0(t, 3H), 1.7(m, 2H), 3.0(t, 2H), 5.2(s, 2H), 7.5-7.9(d, 2H)
06	CH	2,4,5-Cl	H	2-thiopropyl	89-90	1.4(d, 6H), 3.6-4.0 (m, 1H), 4.8(s, 2H), 7.0-7.5(d,2H)
07	CH	2,4,5-Cl	H	2-thiobutyl	72-74	1.0-2.1(m, 8H), 3.5- 4.1(m, 1H), 4.9(s, 2H), 7.0-7.5(d, 2H)
08	CH	2,4,5-Cl	H	2,6-diethylaminophenyl	176-178	1.3(t, 6H), 2.6(q, 4H), 5.0(s, 2H), 7.2-7.9 (m, 5H), 9.5(s, 1H)
09	CH	2,4,5-Cl	H	2-chloroaminophenyl	210-212	4.8(s, 2H), 7.0-7.9 (m, 7H)
10	CH	2,4,5-Cl	H	2-aminobenzothiazolyl	230>	5.2(s, 2H), 7.3-8.2 (m, 7H)
11	CH	2,4,5-Cl	H	2-amino 2-thiazolinyl	205-207	3.3-4.2(m, 4H), 5.0 (s, 2H), 7.2(s, 1H), 7.6(s, 1H)
12	CH	2,4,5-Cl	H	2-amino 5-chlorobenzimidazolyl	225-227	5.2(s, 2H), 7.0-7.9 (m, 6H), 9.0(s, 1H)
13	CH	2,4,6-Cl	H	3-aminopyridyl	120-123	5.1(s, 2H), 8.0(s, 2H), 7.5-8.4(m, 4H), 9.2 (s, 1H)

Table I. Continued

Comp.	H	Xn	R	R'	mp (°C)[a]	NMR data
14	CH	2,4,6-Cl	H	OH	179-182	4.8(s, 2H), 7.9(s, 2H), 9.2(s, 1H)
15	CH	2,4,6-Cl	H	O^-Na^+	250>	4.8(s, 2H), 7.6(s, 2H)
16	CH	2,4,6-Cl	H	O^-K^+	250>	4.8(s, 2H), 7.7(s, 2H)
17	CH	2,4-Cl, 5-CH_3	H	4-chlorobenzylamino	164-166	2.3(s, 3H), 4.5(d, 2H), 4.9(s, 3H), 6.8-7.4 (m, 7H)
18	CH	2,4-Cl, 5-CH_3	H	5-ethylacetoxy 2-aminothiazolyl	130-132	1.2(t, 3H), 2.3(s, 3H), 3.8(s, 2H), 4.1(q, 2H), 4.9(s, 2H), 6.8-7.4(m, 3H), 10.1(s, 1H)
19	CH	2,4,6-Cl, 3-CH_3	H	2-chlorobenzyloxy	96-98	2.41(s, 3H), 4.69(s, 2H), 5.30(s, 2H), 7.35 (m, 5H)
20	CH	2,4-Cl, 5-CH_3	CH_3	4-chlorobenzylamino	134-136	1.6(d, 3H), 4.6(d, 1H), 4.8(q,1H), 6.8-7.5 (m, 6H), 8.9(s, 1H)
21	CH	2,4-Cl, 5-CH_3	CH^3	2-chloro 3-aminopyridyl	142-144	1.7(d, 3H), 4.9(q, 1H), 6.7-8.7(m, 5H), 9.1 (s, 1H)
22	CH	2,4-Cl, 5-CH_3	CH_3	2-methoxy 5-aminopyridyl	127-129	1.7(d, 3H), 3.9(s, 3H), 4.7(q, 1H), 6.6-8.3 (m, 5H), 8.5(s, 1H)
23	CH	2,4,6-Cl, 5-CH_3	H	6-ethoxy 2-aminobenzo thiazolyl	149-150	1.35(t, 3H), 2.41(s, 3H), 4.04(q, 2H), 4.72(s, 2H), 6.85- 7.69(m, 4H), 11.97(s, 1H)
24	CH	2,4-Cl, 5-CH_3	H	OH	141-143	2.2(s, 3H), 4.8(s, 2H), 7.0(s, 1H), 7.5(s, 1H), 10.7(s, 1H)
25	CH	2,4-Cl, 5-CH_3	CH^3	OH	136-137	1.7(d, 3H), 2.4(s, 3H), 5.0(q, 1H), 7.0(s, 1H), 7.4(s, 1H), 10.5 (s, 1H)

[a]The melting points are uncorrected.

Table II. Pharmacological data on synthetic compounds

Compound No.	Acute toxicity (MLD, mg/kg)	Dose (mg/kg)	Antiedema assay (% inhibition)	Dose (mg/kg)	Analgesic assay (% inhibition)	
					Writhing	Tail clip
01	353.6	-	-	100	93.3	6/10[b)]
02	>1000	500	2.8[a)]	250	59.8	5/10
03	>1000	500	25.4	250	50.5	10/10
04	>1000	500	30.0	500	79.3	8/10
05	>1000	500	62.9***	250	62.9	10/10
06	>1000	500	8.0	250	57.2	8/10
07	>1000	500	6.0	250	53.6	8/10
08	>1000	500	8.6	250	69.1	8/10
09	>1000	500	0.0	500	64.9	8/10
10	>1000	500	26.7*	500	72.7	8/10
11	>1000	500	32.4**	500	65.5	8/10
12	>1000	500	48.9**	500	65.5	8/10
13	>500	125	9.9	100	65.5	4/10
14	>500	500	0.0	250	78.4	6/10
15	>500	500	23.6	500	88.1	9/10
16	>500	-	-	100	58.2	4/10
17	>1000	500	18.3	250	4.6	8/10
18	>1000	250	-10.7	500	31.8	10/10
19	>1000	500	26.0	250	20.8	10/10
20	>1000	500	10.5	250	-	10/10
21	>1000	500	10.7	250	29.5	8/10
22	>1000	500	6.2	250	48.6	8/10
23	>1000	250	2.4	250	25.4	10/10
24	>1000	500	42.2**	250	13.8	10/10
25	>1000	500	27.2*	250	30.6	8/10
Hydrocortisone	-	100	51.9**	-	-	-
Aminopyrine	-	-	-	200	78.4	4/10

[a)]Percent inhibition of increase in paw edema volume at 4 hr after the irritant injection
[b)]No. of mice responded/No. used
Significantly different from the control : *P>0.05, **P>0.01, ***P>0.001

test on carrageenin edema, acetic acid writhing syndrome and response on tail clip pressure, and the results were summarized in Table II. The anti-inflammatory effect was measured from 1 hr upto 5 hr after the irritant injection, but only percent inhibition of increase in edema volume at 4 hr was indicated in Table II for comparison of anti edema potencies.

A strong anti-inflammatory activity was observed in compound **05** which exhibited significant inhibition throughout 1-5 hr (46.8%-62.9% inhibition) with a single dose of 500 mg/kg, p.o. Moderate but significant anti-edematous activities were also observed in compounds **11**, **12** and **24** whereas relatively weak edema inhibition were observed in compounds **10** and **25**.

Thus the most striking facet of the structure-activity relationships in anti-inflammatory actions to emerge is that in 2,4,5-trichlorophenoxy derivatives, a high anti-edematous potency is associated with the presence of 1-thiopropyl moiety and a moderate potency with 2-amino thiazolinyl and 2-amino-5-chloro benzimidazolyl moieties. Very weak anti-edematous activities were observed in 2,4-dichloro-5-methyl phenoxy derivatives, but interestingly enough, compounds with OH^- substitution at R' position such as **24** and **25** exhibited significant anti-edematous activities. Substitution with methyl moiety at R' position caused decrease in anti-edema activity. More or less significant inhibition of writhing syndrome was observed in almost all of trichlorophenoxy derivatives , but significant inhibition of response in tail clip pressure was observed only in compounds **01**, **02**, **13** and **16** which showed more or equipotent activity to aminopyrine (200 mg/kg). These results clearly indicate that 3 or 4-aminopyridyl moiety at R' position contributed to the manifestation of analgesic activity. Interestingly, compound **16** which possessed OK^+ at R' position exhibited significant analgesic activity. Writhing syndrome is known to be rather non-specific responses and thus various drugs such as traquilizers and other than analgesic drugs also shows anti-writhing activity (Whittle, 1964). The anti-writhing activities elicited by trichlorophenoxy derivatives in the present experiments may not be considered as true analgesic responses, although their true mechanism of action is not known yet. Among trichloro phenoxy analogues, 4-amino pyridyl substitution contribute to the acute toxicity, its LD_{50} values, being 353,6 mg/kg orally in compound **01**. Further synthesis of phenoxyalkanoic acid derivatives and elucidation on their pharmacological activities are under investigation which will be reported elsewhere.

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