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Anti-inflammatory properties of a series of phenyl- and phenoxy-alkanoic acids

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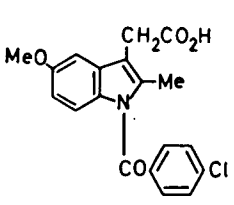
Some *o*-benzamidophenoxyacetic, phenylalkanoic and phenoxy-alkanoic acids have been synthesized. Anti-inflammatory activity was measured by the phenylbenzoquinone writhing test in mice and the rat foot oedema test. *Meta-* and *para*-substitutions in the benzamido-ring, promoting lipid solubility, enhanced the potency, whereas substitution with polar groups reduced it. Further phenyl ring substitution in the 2-(3,4-dichlorobenzamido)phenoxyacetic acids only slightly affected the potency. Side-chain modifications did not increase the activity on the three substituted phenoxyacetic acids chosen. Two phenylpropionic acids showed a good order of activity but the respective cinnamic acids were virtually inactive. From the investigations 2-(3,4-dichlorobenzamido)phenoxyacetic acid (SNR. 1804) was selected for further studies is now undergoing clinical evaluation.

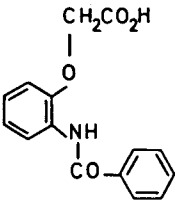
The anti-inflammatory activity of indomethacin (I) (Shen, 1963) promoted a search for this activity in related compounds in which the indole nucleus was replaced with simpler aromatic systems.

By analogy with the plant growth hormones, where activity is found both in 3-indoleacetic acids and in phenoxyacetic acids, it seemed reasonable to consider the phenoxyacetic structure as a basis for the design of potential anti-inflammatory agents, and Northover & Subramanian (1961) had already demonstrated such activity in some substituted α-phenoxypropionic acids.

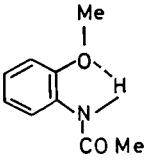
The introduction of a benzamido-group in the *ortho*-position of a phenoxyacetic acid would yield compound (II) with some formal resemblance to the structure of indomethacin (I).



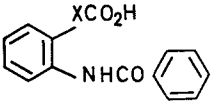
I



II



III



IV

That this structural comparison might have some validity was supported by studies by Ungnade (1954) on the ultraviolet absorption spectra of substituted acetanilides. Ungnade examined the spectra of *o*-, *m*-, and *p*-alkyl, halo- and methoxy substituted acetanilides. In these, the introduction of such groups *ortho* led to a decrease in intensity of absorption and a shift towards lower wavelength (compared with

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corresponding *m*- or *p*-compounds). This was interpreted as a steric effect in which the bulky *ortho* substituent twists the acetamido group out of the plane of the ring. Such an effect was not observed with *o*-methoxyacetanilide, and Ungnade suggested that the formation of a weak hydrogen bond stabilized the compound in the non-hindered conformation (III).

A series of *o*-benzamidophenoxyacetic acids was prepared and many of these showed anti-inflammatory activity. To investigate further the structural features necessary for activity, compounds with the general formula (IV) were prepared including phenylacetic ($X = CH_2$), β-phenylpropionic ($X = CH_2CH_2$), and cinnamic ($X = CH{=}CH$) acids, as well as extensions of the phenoxyacetic ($X = OCH_2$) series to include α- and ω-phenoxyalkanoic acids.

CHEMISTRY EXPERIMENTAL

The compounds of Tables 1 and 2 were prepared by several methods, which are illustrated in the following examples.

Method A

2-(o-*Chlorobenzamido*)*phenoxyacetic acid* (*cpd no.* 3). *o*-Nitrophenoxyacetic acid (39·2 g; 0·2 mol) was dissolved in aqueous NaOH (250 ml; 0·8N) and platinum oxide catalyst (0·5 g) was added. The solution was hydrogenated at room temperature and pressure, the solution was filtered from catalyst and aqueous NaOH (40 ml; 5N) added. To this solution was added *o*-chlorobenzoyl chloride (35·0 g; 0·2 mol), with stirring at 10° during 15 min. The solution was stirred for a further 2 h at 10–20° and extracted with $CHCl_3$ (2 × 200 ml). Evaporation gave an oil which was extracted with boiling di-n-butyl ether (300 ml). On cooling crystals, m.p. 123–5° (26·2 g), were obtained. The crude material was recrystallized from di-n-butyl ether (200 ml) and then from ethyl acetate (70 ml) to yield pure cpd no. 3 as colourless crystals, m.p. 143–4° (10·1 g; 17%). ($C_{15}H_{12}ClNO_4$) C, H, N.

Method B

2-(3,4-*Dichlorobenzamido*)-4-*methoxyphenoxyacetic acid* (*cpd no.* 34). 2-*Chloroacetamido*-4-*methoxyphenol.* To a solution of 2-amino-4-methoxyphenol (46·0 g; 0·33 mol) in dry Me_2CO (300 ml) containing Et_3N (33·5 g; 0·33 mol) was added chloracetyl chloride (37·5 g; 0·33 mol) dropwise with stirring at 5–10° during 30 min. The solution was stirred for a further 30 min at 10° and the Me_2CO removed under reduced pressure. H_2O (1 litre) was added to the solid which was filtered, washed (H_2O) and dried to give the product m.p. 173–6·5° (70·3 g; 98·5%). An analytical sample was prepared by two further recrystallizations from EtOH–H_2O (2:1 v/v) to give 2-chloroacetamido-4-methoxyphenol as pale yellow needles, m.p. 183–4°. ($C_9H_{10}ClNO_3$) C, H, N.

2,3-*Dihydro*-6-*methoxy*-1,4-*benzoxazin*-3-*one.* 2-Chloroacetamido-4-methoxyphenol (53·9 g; 0·2 mol) was dissolved in boiling EtOH (420 ml) and aqueous NaOH (560 ml; N) was added. The solution was boiled under reflux for 15 min and neutralized to pH 7 by the addition of dilute HCl (280 ml; N). After standing overnight at 5° the solid was filtered, washed with water and dried. The crude product was recrystallized from benzene (charcoal) to give 2,3-dihydro-6-methoxy-1,4-benzoxazin-3-one as colourless needles, m.p. 168–9° (31·3 g; 70%). ($C_9H_9NO_3$) C, H, N.

2-(3,4-*Dichlorobenzamido*)-4-*methoxyphenoxyacetic acid.* 2,3-Dihydro-6-methoxy-1,4-benzoxazine-3-one (8·95 g; 0·05 mol) was boiled under reflux for 2 h with aqueous NaOH (50 ml; 5N). To the solution was added H_2O (50 ml) and dilute HCl (40 ml; 5N) and the solution was cooled to 10°. A solution of 3,4-dichlorobenzoyl chloride (10·47 g; 0·05 mol) in Et_2O (50 ml) was added during 1½ h at 10° with stirring at the same time maintaining pH 7–9 by the simultaneous addition of aqueous NaOH (10 ml; 5N). During the course of the reaction the sodium salt precipitated and a further 150 ml H_2O was added to facilitate stirring. The solution was stirred at 10–20° for a further 1 h and the sodium salt was filtered, dissolved in Me_2CO–H_2O (150 ml, 1:1 v/v) and acidified to pH 3 with HCl. The solid was filtered, washed (H_2O) and dried (13·2 g), m.p. 182–7°. The crude product was recrystallized from methyl ethyl ketone to give pure cpd no. 34 as colourless needles, m.p. 195–6° (7·7 g; 42%). An analytical sample was prepared by a further recrystallization from methyl ethyl ketone to give colourless needles, m.p. 196–7°. ($C_{16}H_{13}Cl_2NO_5$) C, H, N.

Method C

γ-[2-(m-*Trifluoromethylbenzamido*)-4-*chlorophenoxy*]*butyric acid* (*cpd no.* 47). 2-(m-*Trifluoromethylbenzamido*)-4-*chlorophenol.* To a solution of 2-amino-4-chlorophenol (7·16 g; 0·05 mol) in tetrahydrofuran (THF) (100 ml), containing *NN*-dimethylaniline (5·85 g; 0·05 mol) was added a solution of *m*-trifluoromethylbenzoyl chloride (10·5 g; 0·05 mol) in THF (50 ml) at 5–10° with stirring during 45 min. The solution was stirred for a further 1 h at 10° and the THF removed under reduced pressure. The resulting oil was poured into H_2O (500 ml) containing concentrated HCl (50 ml) and the precipitate was filtered and washed (H_2O). The crude solid was dissolved in Me_2CO (200 ml) and added to aqueous NaOH (1 litre; 0·125N). To this solution was added solid CO_2 with occasional stirring to pH 6. The precipitated 2-(*m*-trifluoromethylbenzamido)-4-chlorophenol was filtered, washed with water (100 ml) and dried, m.p. 190–1° (13·9 g; 88%). An analytical sample was prepared by a further recrystallization from EtOH–H_2O (1:1 v/v) to give colourless needles, m.p. 201–2°. ($C_{14}H_9ClF_3NO_2$) C, H.

γ-(2-(m-*Trifluoromethylbenzamido*)-4-*chlorophenoxy*)*butyric acid.* Sodium (0·92 g; 0·04 mol) was dissolved in n-BuOH (200 ml), and to the warm solution was added 2-(*m*-trifluoromethylbenzamido)-4-chlorophenol (12·62 g; 0·04 mol). To this solution was added ethyl γ-chlorobutyrate (6·02 g; 0·04 mol) and sodium iodide (6·0 g; 0·04 mol) and the solution heated under reflux for 8½ h. After cooling and filtration, the solvent was removed under reduced pressure to give the crude ester. This was dissolved in EtOH (200 ml) containing aqueous NaOH (9 ml; 5N) and boiled under reflux for 1 h. The solution was cooled, poured into dilute HCl (20 ml; 5N), and the solid filtered. The crude product was dissolved in EtOAc (200 ml) and extracted three times with dilute NH_4OH (200 ml, 2 × 100 ml). The combined ammoniacal extract was acidified with concentrated HCl with cooling. The solid was filtered and washed (H_2O), dissolved in hot HOAc (100 ml) with charcoal, filtered, and H_2O (200 ml) added gradually with heating. On cooling, cpd no. 47 was obtained as colourless needles, m.p. 148–50° (4·82 g; 30%). An analytical sample was prepared by a further recrystallization from HOAc–H_2O (1:1 v/v) to give colourless needles, m.p. 150–1°. ($C_{18}H_{15}ClF_3NO_4$) C, H, N.

Method D

β-(2-(3,4-Dichlorobenzamido)-4-methylphenoxy)propionic acid (*cpd no.* 43). *β-(4-Methyl-2-nitrophenoxy)propionic acid.* To a solution of 4-methyl-2-nitrophenol (51·0 g; 0·33 mol) in aqueous NaOH (200 ml; 1·67N) at 96° was added β-propiolactone (24 g; 0·33 mol) dropwise with stirring during 30 min at 96–102°. The mixture was stirred for a further 20 min at 96°, cooled and acidified with dilute HCl. An orange oil separated, which was extracted with Et_2O (3 × 100 ml). The Et_2O solution was extracted with saturated $NaHCO_3$ solution and the aqueous layer acidified with HCl to pH 3. The yellow solid was filtered, washed and dried. The crude product was recrystallized from HOAc–H_2O (1 : 2 v/v) to give pure β-(4-methyl-2-nitrophenoxy)-propionic acid as yellow needles, m.p. 111–2° (24·2 g; 32%). ($C_{10}H_{11}NO_5$) C, H, N.

β-[2-(3,4-Dichlorobenzamido)-4-methylphenoxy]propionic acid. A solution of β-(4-methyl-2-nitrophenoxy)propionic acid (11·25 g; 0·05 mol) in aqueous NaOH (200 ml; 0·25N) was hydrogenated over 3% Pd/C (1 g), at room temperature and atmospheric pressure. The catalyst was filtered and the filtrate was treated with dilute HCl (10 ml; 5N). The H_2O was removed under reduced pressure and the resulting white solid was dried, triturated with THF (220 ml) and the NaCl filtered. To the filtrate was added *NN*-dimethylaniline (13·3 g; 0·11 mol) followed by 3,4-dichlorobenzoyl chloride (10·5 g; 0·05 mol) in THF (30 ml) dropwise over 30 min at 10–15° with stirring. The mixture was stirred for a further 2 h at 10–20°, the solvent removed under reduced pressure and the residue dissolved in EtOH (50 ml). The EtOH solution was added with stirring to dilute HCl (500 ml; 0·4N) and the solid was filtered, washed (H_2O) and dried. The crude product (14 g) was recrystallized from HOAc–H_2O (1 : 1 v/v) to give β-[2-(3,4-dichlorobenzamido)-4-methylphenoxy]-propionic acid as colourless needles, m.p. 158–9° (9·5 g; 52%). ($C_{17}H_{15}Cl_2NO_4$) C, H, N.

Method E

4-Chloro-2-(m-trifluoromethylbenzamido)phenylacetic acid (*cpd no.* 50). *Ethyl-4-chloro-2-nitrophenylacetate.* 4-Chloro-2-nitrophenylacetic acid [lit. m.p. 166–8°—Wright & Collins, 1956] (38·7 g; 0·18 mol) in EtOH (400 ml) containing conc. H_2SO_4 (1 ml) was boiled under reflux for 2½ h. The solution was poured onto crushed ice (1000 g), the suspension Et_2O extracted, the extracts combined and washed with saturated $NaHCO_3$ solution, H_2O, and then dried (Na_2SO_4). Removal of the Et_2O under reduced pressure left a solid which was crystallized from EtOH (40 ml) by cooling to 5° to give ethyl 4-chloro-2-nitrophenyl acetate (32·6 g; 74%), m.p. 41–2°. Recrystallization from EtOH gave material of m.p. 42–43°. ($C_{10}H_{10}ClNO_4$) C, H, N.

Ethyl-4-chloro-2-(m-trifluoromethylbenzamido)phenylacetate. Ethyl-4-chloro-2-nitrophenylacetate (10 g; 0·041 mol) was dissolved in EtOH (140 ml) and catalytically reduced over platinum oxide (0·1 g). The catalyst was filtered and solvent removed under reduced pressure and the residual oil was dissolved in Me_2CO (100 ml) containing Et_3N (4·15 g; 0·041 mol) and cooled to 5°. A solution of *m*-trifluoromethylbenzoyl chloride (3·4 g; 0·040 mol) in Me_2CO (50 ml) was added slowly to the amine solution over 1 h, and the mixture then boiled under reflux for 1 h, and left overnight. The resulting solution was poured onto crushed ice (1500 g), the mixture extracted with Et_2O, the extracts combined, washed with H_2O and dried (Na_2SO_4). The Et_2O was removed under reduced pressure to leave a red oil (15·5 g) which was

dissolved in light petroleum (60–80°) (50 ml) and cooled to give a buff solid. This was recrystallized from EtOAc–light petroleum (60–80°) (1:4 v/v) to give ethyl 4-chloro-2-(*m*-trifluoromethylbenzamido)phenylacetate (4·5 g; 28·5%), m.p. 83–85°. Recrystallization from EtOAc-light petroleum (1:4 v/v) gave pink needles, m.p. 86–88°. ($C_{18}H_{15}ClF_3NO_3$) C, H.

4-*Chloro*-2-(m-*trifluoromethylbenzamido*)*phenylacetic acid* (*cpd no.* 50). Ethyl-4-chloro-2-(*m*-trifluoromethylbenzamido)phenylacetate (4·18 g; 0·0108 mol) was dissolved in Me_2CO (50 ml) and aqueous KOH (7 ml; 1·18N) added with stirring, keeping the temperature below 10°. The reaction mixture was left overnight and the Me_2CO removed under reduced pressure to leave red oil. H_2O (60 ml) was added and the mixture extracted with Et_2O. The aqueous solution was acidified with HCl (5N) to give a buff coloured solid (3·61 g). Recrystallization from Me_2CO (charcoal) gave cpd no. 50 (3·08 g; 86%), m.p. 185–6°. Recrystallization from Me_2CO–light petroleum (60–80°) (30 ml; 1:1 v/v) gave material m.p. 187–188°. ($C_{16}H_{11}ClF_3NO_3$) C, H, N.

Method F

β-[4-*Chloro*-2-(m-*trifluoromethylbenzamido*)*phenyl*]*propionic acid* (*cpd no.* 53). 7-Chloro-3,4-dihydrocarbostyril (C.A. 66, 37125h, (1967) (8·35 g; 0·046 mol) was boiled under reflux for 16 h with aqueous NaOH (50 ml; 5N). The solution was diluted with H_2O (50 ml), cooled to 10°, and HCl (5N) added dropwise to bring the pH to 8–9. The mixture was treated with a solution of *m*-trifluoromethylbenzoyl chloride (9·3 g; 0·046 mol) in Me_2CO (15 ml) maintaining the temperature at 10° and the pH at 8–9 by the simultaneous addition of NaOH (N). After a further 2 h stirring the mixture was filtered, extracted with Et_2O (2 × 50 ml) and the aqueous phase acidified to pH 2 with conc. HCl. The precipitated solid was filtered, washed (H_2O) and dried to give a solid (5·65 g) which after recrystallization from Me_2CO–H_2O (2:1 v/v) gave cpd no. 53 as white plates (3·54 g; 21%), m.p. 158–60°. ($C_{17}H_{13}ClF_3NO_3$) C, H, N.

Method G

4-*Chloro*-2-(m-*trifluoromethylbenzamido*)*cinnamic acid* (*cpd no.* 56). 4-*Chloro*-2-*nitrocinnamic acid.* 4-Chloro-2-nitrobenzaldehyde (15·7 g; 0·085 mol) and malonic acid (17·6 g; 0·169 mol) were added to pyridine (30 ml) containing piperidine (2 ml). The mixture was heated for 3 h on a steam bath, cooled and poured into water (300 ml) containing conc. HCl (100 ml). The precipitated solid was filtered, washed with water and dried to give 4-chloro-2-nitrocinnamic acid (13·8 g; 72%). A portion recrystallized from benzene–light petroleum (40–60°) (1:1 v/v) gave material with m.p. 214° (Van der Lee, 1926).

2-*Amino*-4-*chlorocinnamic acid.* A solution of 4-chloro-2-nitrocinnamic acid (17·64 g; 0·078 mol) in NH_4OH (170 ml; 5N) was added dropwise with stirring to a solution of ferrous sulphate heptahydrate (174 g; 0·625 mol) in water (260 ml). NH_4OH solution (65 ml) was added and the mixture heated for 10 min on a steam bath, cooled and centrifuged. The supernatant was removed and the solid stirred with NaOH (500 ml; N) and again centrifuged. The supernatants were combined, concentrated to 400 ml under reduced pressure, the pH adjusted to 5 with HCl (5N) and the precipitate filtered, dissolved in NH_4OH (5N) and reprecipitated with HOAc to give material (11·67 g; 76% yield), m.p. 203–4°. Recrystallization from Et_2OH

gave yellow needles, m.p. 209–10°. A satisfactory analysis for 2-amino-4-chlorocinnamic acid could not be obtained.

4-*Chloro*-2-(m-*trifluoromethylbenzamido*)*cinnamic acid* (*cpd no.* 56). A solution of *m*-trifluoromethylbenzoyl chloride (5·3 g; 0·025 mol) in Me_2CO (30 ml) was added dropwise with stirring to a solution of 2-amino-4-chlorocinnamic acid (5·0 g; 0·025 mol) aqueous NaOH (40 ml; 0·625N). The pH of the reaction was maintained at 8 by the dropwise addition of an aqueous NaOH (40 ml; 0·625N). After being stirred for 30 min the reaction mixture was adjusted to pH 2 with conc. HCl. The precipitate was filtered, washed (H_2O), dried, and recrystallized from HOAc to give cpd no. 56 (0·31 g; 35% yield), m.p. 227–8°. ($C_{17}H_{11}ClF_3NO_3$) C, H, N.

PHARMACOLOGY EXPERIMENTAL

Methods

Acute toxicity. Male albino, Smith and Nephew Research (SNR) strain mice, 25–30 g were used, 4 animals per dose level. Approximate LD50 values were determined by inspection from mortalities occurring within 3 days of oral or intraperitoneal administration.

Phenylbenzoquinone (*PBQ*) *writhing test in mice.* Female albino SNR strain mice, four to six weeks old were used. A modification of the method of Siegmund, Cadmus & Lu (1957) was used. Mice were injected with PBQ 35 min after the oral administration of the anti-inflammatory compound. The mice were then observed during the 5 min period at which maximum writhing rate occurred in control animals.

(*a*) Screening test. This was a sequential test based upon the quantal responses of up to four successive groups of six mice. All compounds were screened at an oral dose of 2/5 LD50. All compounds which protected four or more mice out of twelve were examined further by a potency estimation.

(*b*) Potency. The relative potencies of the accepted compounds were determined using the method of Hendershot & Forsaith (1959) in which the dose which reduces the writhing rate by 50% is obtained from dose response curves constructed using groups of 10 mice.

Rat foot oedema (*carrageenan*) *test.* A modification of the method of Winter, Risley & Nuss (1962) was used. The initial foot volume of the rats was determined volumetrically. 0·1 ml of a suspension of carrageenan (1% in normal saline) was injected subcutaneously into the plantar region of the right hind paw 1 h after the test compounds at 50 mg/kg had been administered orally. Three h later the foot volume was again measured and the volume of oedema determined. Results were expressed as percentage inhibition related to a control oedema volume.

The oedema volume in control and treated animals was compared using students *t*-test.

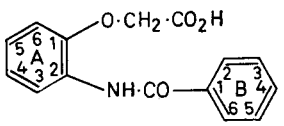
Compound no. 12 (SNR 1804; clamidoxic acid) was selected for further evaluation by the mouse tail pinch method of Bianchi & Franchescini (1954), a modified cotton wool pellet granuloma test (Winter & Porter, 1957), fever induction (Brownlee, 1939) and gastric haemorrhage in guinea-pigs (Anderson, 1963).

RESULTS AND DISCUSSION

Although the phenylbenzoquinone writhing syndrome is known to be non-specific in its response to drugs, results from this test were used to guide the synthetic

program for the following reasons. Evidence from the inflamed rat paw test, and other anti-inflammatory tests made on selected compounds suggested that in this series of compounds the activity being detected by the PBQ assay was in fact anti-inflammatory activity. Furthermore, in our hands the test has been found to yield reproducible results and good dose-response lines even with comparatively weak anti-inflammatory agents, whereas we have found it difficult to obtain reproducible quantitative data with the rat paw test.

Table 1. *2-Benzamido-phenoxyacetic acids.* Compounds were prepared by method B, except nos. 1 and 3 which were prepared by method A. All compounds had C, H, N analyses within the usual limits. All compounds except nos. 19, 21, 28 and 29 had oral LD50 doses of 1000 mg/kg or greater; those for 19, 21, 28 and 29 were 750, 750, 600 and 700 mg/kg respectively.



Comp. No.	Subt. Ring A	Subt. Ring B	m.p. °C	Formula	PBQ test (oral) Mice protected at 2/5 LD50	PBQ test (oral) 50% reduction in writhing dose (mg/kg)	Rat foot test*
1	—	—	164	$C_{15}H_{13}NO_4$	4/12	140	19—
2	—	4-Me	198	$C_{16}H_{15}NO_4$	19/24	40	22—
3	—	2-Cl	143	$C_{15}H_{12}ClNO_4$	0/6	NT	0
4	—	3-Cl	161	$C_{15}H_{12}ClNO_4$	15/18	40	0
5	—	4-Cl	215	$C_{15}H_{12}ClNO_4$	9/18	100	0
6	—	3-Br	158	$C_{15}H_{12}BrNO_4$	10/12	40	24+
7	—	4-Br	210	$C_{15}H_{12}BrNO_4$	11/12	75	11—
8	—	4-F	183	$C_{15}H_{12}FNO_4$	11/12	40	8—
9	—	4-I	192	$C_{15}H_{12}INO_4$	5/6	35	0
10	—	2,4-Cl_2	196	$C_{15}H_{11}Cl_2NO_4$	0/6	NT	0
11	—	2,5-Cl_2	188	$C_{15}H_{11}Cl_2NO_4$	0/6	NT	0
12	—	3,4-Cl_2	218	$C_{15}H_{11}Cl_2NO_4$	12/12	31	39+
13	—	3,5-Cl_2	237	$C_{15}H_{11}Cl_2NO_4$	10/12	15	2—
14	—	3-CF_3	160	$C_{16}H_{12}F_3NO_4$	14/18	29	0
15	—	2-OH	162	$C_{15}H_{13}NO_5$	8/12	49	0
16	—	4-OH	162	$C_{15}H_{13}NO_5$	3/12	NT	0
17	—	4-$MeSO_2$	177	$C_{16}H_{15}NO_6S$	2/12	NT	0
18	4-Me	—	147	$C_{16}H_{15}NO_4$	8/12	50	8—
19	4-Me	4-Cl	174	$C_{16}H_{14}ClNO_4$	11/12	11	5—
20	4-Me	3,4-Cl_2	218	$C_{16}H_{13}Cl_2NO_4$	12/12	40	37+
21	4-Me	3-CF_3	161	$C_{17}H_{14}F_3NO_4$	12/12	27	0
22	5-Me	3,4-Cl_2	233	$C_{16}H_{13}Cl_2NO_4$	4/12	49	0
23	4-Et	3,4-Cl_2	209	$C_{17}H_{15}Cl_2NO_4$	12/12	19	29+
24	4-CH<(Me, Et)	3,4-Cl_2	138	$C_{19}H_{19}Cl_2NO_4$	12/12	38	11—
25	4-CMe_3	3,4-Cl_2	182	$C_{19}H_{19}Cl_2NO_4$	19/24	50	0
26	3,5-Me_2	3,4-Cl_2	229	$C_{17}H_{15}Cl_2NO_4$	6/18	62	0
27	4,5-Me_2	3,4-Cl_2	235	$C_{17}H_{15}Cl_2NO_4$	7/12	95	0
28	4-Cl	—	166	$C_{15}H_{12}ClNO_4$	15/18	85	46+
29	4-Cl	4-Cl	164	$C_{15}H_{11}Cl_2NO_4$	16/24	150	35±
30	4-Cl	3,4-Cl_2	208	$C_{15}H_{10}Cl_3NO_4$	12/12	29	56+
31	4-Cl	3-CF_3	159	$C_{16}H_{11}ClF_3NO_4$	11/12	32	49+
32	4-Cl	4-MeO	195	$C_{16}H_{14}ClNO_5$	15/24	100	24+
33	4-Br	3,4-Cl_2	200	$C_{15}H_{10}BrCl_2NO_4$	7/12	32	24±
34	4-MeO	3,4-Cl_2	196	$C_{16}H_{13}Cl_2NO_5$	7/12	37	14±
35	5-MeO	4-Cl	207	$C_{16}H_{14}ClNO_5$	11/12	67	0
36	5-MeO	3,4-Cl_2	222	$C_{16}H_{13}Cl_2NO_5$	8/12	77	0
37	4-OH	3,4-Cl_2	254	$C_{15}H_{11}Cl_2NO_5$	6/12	130	0
38	5-OH	3,4-Cl_2	221	$C_{15}H_{11}Cl_2NO_5$	0/12	NT	0

* Oral, % reduction in oedema at 50 mg/kg.
NT = not tested.

Anti-inflammatory activity was found in most of the basic structural types examined. The unsubstituted *o*-benzamidophenoxyacetic acid (cpd 1, Table 1) showed activity of about the same order as aspirin or phenylbutazone, and the most active of the substituted derivatives (cpd 13, 19, 50, 52, 53) were approximately ten times as potent.

Substitution in Ring B

The introduction of a single halogen atom in Ring B in the *m*- and *p*-positions generally increased potency up to three fold (Table 1, cpd 4, 5, 6, 7, 8, 9) whereas similar substitution in the *o*-position destroyed activity (cpd 3). In a series of dichloro-derivatives the presence of a Cl atom in the *o*-position again yielded inactive compounds (cpd 3, 10, 11) whereas the 3,4- and 3,5-compounds (12, 13) had 10 and 4 times the potency of the unsubstituted parent. Of other substituents examined, those promoting lipid solubility yielded compounds with increased activity, whereas polar substituents gave inactive compounds (16, 17). A simple regression analysis was made on the first 17 compounds of Table 1 excluding those with an *o*-substituent (cpd 3, 10, 11, 15), using log 1/ID50 as an expression of biological activity, against the Hansch substituent constant, π (Hansch & Fujita, 1964). The equation obtained was $\log 1/ID50 = 0{\cdot}55\pi - 2{\cdot}14$ with correlation coefficient = 0·87.

Substitution in Ring A

Only two compounds (18, 28) were prepared with substitution (Me, Cl) in Ring A alone. Both of these were more potent than the unsubstituted parent, suggesting that here also groups promoting lipid solubility yielded compounds with increased potency. Ring A variations were studied more extensively in a group of compounds with 3,4-Cl_2 substitution in Ring B. The introduction of further substituents (alkyl, halogen, methoxy) affected activity only slightly and generally adversely, but again the introduction of a hydroxyl group (cpd 37, 38) reduced activity markedly.

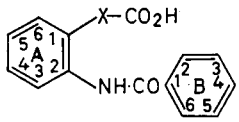
Modification of side-chain

To examine the effect of variations in side-chain linking Ring A to the carboxyl group, three representative substituted phenoxyacetic acids were chosen (cpd 12, 20, 31), as bases for modification. α-Alkyl substitution had little effect on activity (cpd 39–41) (Table 2) whereas lengthening of the side-chain, as in the β-phenoxypropionic acids (cpd 42–44) and γ-phenoxybutyric acids (cpd 45–47) caused a significant reduction in activity, the magnitude of the effect depending on the individual ring substituents.

Removal of the ether oxygen atom yielded phenylacetic (cpd 48–50) and phenylpropionic (cpd 51–53) acids. Two of the latter compounds, in which the "chain length" was similar to that of the phenoxyacetic acids, were among the most potent of the series. In contrast, the cinnamic acid analogues (cpd 54–56) were virtually inactive. These latter compounds would be expected to have a relatively rigid *trans*-configuration thus fixing the position of the carboxyl group relative to the planar ring skeleton, and lack of activity suggests that the position and overall separation of the carboxyl group relative to the ring system has a considerable bearing on the level of potency within this class of compound.

Clamidoxic acid was shown, using the mouse tail pinch method of Bianchi & Franchescini (1954) not to possess central anti-nociceptive activity and it seems reasonable

Table 2. *2-Benzamidophenylalkanoic and -phenoxyalkanoic acids.* All compounds had C, H, N analyses within the usual limits. All compounds except nos. 41, 50 and 56 had LD50 doses of 1000 mg/kg or greater; those for 41, 50 and 56 were 700, 750 and 250 mg/kg respectively.



Compound No.† method	Subt. Ring A	Subt. Ring B	X	m.p.	Formula	PBQ test (oral) Mice protected at 2/5 LD50	PBQ test (oral) 50% reduction writhing dose (mg/kg)	Rat foot test‡
39 B*	—	3,4-Cl_2	-O-CH(Me)	171°	$C_{16}H_{13}Cl_2NO_4$	8/12	47	0
40 B*	4-Me	3,4-Cl_2	-O-CH(Me)	175°	$C_{17}H_{15}Cl_2NO_4$	12/12	39	0
41 C	4-Cl	3-CF_3	-O-CH(Et)	132°	$C_{18}H_{15}ClF_3NO_4$	11/12	40	19−
42 D	—	3,4-Cl_2	-O-$[CH_2]_2$-	160°	$C_{16}H_{13}Cl_2NO_4$	11/12	55	0
43 D	4-Me	3,4-Cl_2	-O-$[CH_2]_2$-	158°	$C_{17}H_{15}Cl_2NO_4$	8/12	120	10−
44 D†	4-Cl	3-CF_3	-O-$[CH_2]_2$-	197°	$C_{17}H_{12}ClF_3NO_4$	2/12	NT	9−
45 C	—	3,4-Cl_2	-O-$[CH_2]_3$-	126°	$C_{17}H_{15}Cl_2NO_4$	16/18	80	0
46 C	4-Me	3,4-Cl_2	-O-$[CH_2]_3$-	150°	$C_{18}H_{17}Cl_2NO_4$	8/12	40	17−
47 C	4-Cl	3-CF_3	-O-$[CH_2]_3$-	150°	$C_{18}H_{14}ClF_3NO_4$	16/18	70	0
48 E	—	3,4-Cl_2	-CH_2-	189°	$C_{16}H_{11}Cl_2NO_3$	13/18	80	0
49 E	4-Me	3,4-Cl_2	-CH_2-	178°	$C_{16}H_{13}Cl_2NO_3$	11/12	40	19−
50 E	4-Cl	3-CF_3	-CH_2-	188°	$C_{16}H_{11}ClF_3NO_3$	11/12	14	7−
51 F	—	3,4-Cl_2	-CH_2-CH_2-	185°	$C_{16}H_{13}Cl_2NO_3$	2/12	NT	12−
52 F	4-Me	3,4-Cl_2	-CH_2-CH_2-	183°	$C_{17}H_{15}Cl_2NO_3$	12/12	6	37+
53 F	4-Cl	3-CF_3	-CH_2-CH_2-	159°	$C_{17}H_{12}ClF_3NO_3$	11/12	15	68+
54 G	—	3,4-Cl_2	-CH=CH-	275°	$C_{16}H_{11}Cl_2NO_3$	4/12	160	0
55 G	4-Me	3,4-Cl_2	-CH=CH-	263°	$C_{17}H_{13}Cl_2NO_3$	2/12	NT	0
56 G	4-Cl	3-CF_3	-CH=CH-	227°	$C_{17}H_{11}ClF_3NO_3$	3/12	NT	0
	Aspirin					—	110	27+
	Phenylbutazone					—	100	26+
	Indomethacin					—	1·3	27+§

*α-Chloropropionylchloride was used for these two compounds.
† The intermediate 3-(4-chloro-2-nitrophenoxy)-propionic acid was reduced with sodium dithionite instead of catalytically.
‡ Oral, % reduction in oedema at 50 mg/kg. *P* values for *t*-tests on rat foot oedema data are as follows: +=$P<0{\cdot}05$ ± = $P<0{\cdot}1$ to $>0{\cdot}05$ − = $P>0{\cdot}1$.
§ Indomethacin dose = 1·0 mg/kg orally.
NT = not tested.

to suggest that its activity in the PBQ writhing test is due to its anti-inflammatory activity. In this test clamidoxic acid was found to be more active than phenylbutazone, acetylsalicylic acid and flufenamic acid and less active than indomethacin.

The order of activity of clamidoxic acid was also established in the carrageenan rat foot oedema test where it was shown to be more active than acetylsalicylic acid, equivalent to phenylbutazone and much less active than indomethacin. Clamidoxic acid maintained its activity in the carrageenan foot test in adrenalectomized rats, indicating that its anti-inflammatory activity is not mediated via the adrenal cortex.

The acute response to clamidoxic acid in the carrageenan rat foot test was not modified by pretreatment of the animals with clamidoxic acid.

A modification of the cotton wool pellet granuloma test (Winter & Porter, 1957) was used to grade the activity of clamidoxic acid which was approximately equivalent to phenylbutazone and less active than indomethacin.

Clamidoxic acid was half as effective as aspirin and one quarter as active as phenylbutazone as an antipyretic agent in the fever test in rats (Brownlee, 1939).

Clamidoxic acid does not produce gastric haemorrhages in guinea-pigs to the same extent as acetylsalicylic acid and indomethacin (Anderson, 1963) and was significantly less toxic than acetylsalicylic acid in subacute toxicity tests in rats.

This compound was therefore selected to undergo intensive pharmacological and toxicological testing and is now undergoing clinical evaluation in rheumatic and allied inflammatory conditions.

Table 3. *Intermediates not listed in the literature*

Intermediate for compound	Compound	m.p.	Molecular formula	Analyses
23	2,3-Dihydro-6-ethyl-1,4-benzoxazin-3-one	160–1°	$C_{10}H_{11}NO_2$	C, H, N
	2-Chloracetamido-4-ethylphenol	153–4°	$C_{10}H_{12}ClNO_2$	C, H, N
24	2,3-Dihydro-6-s-butyl-1,4-benzoxazin-3-one	85–6°	$C_{12}H_{15}NO_2$	C, H, N
	2-Chloracetamido-4-s-butylphenol	193–4°	$C_{12}H_{16}ClNO_2$	C, H, N
25	2,3-Dihydro-6-t-butyl-1,4-benzoxazin-3-one	151–2°	$C_{12}H_{15}NO_2$	C, H, N
	2-Chloracetamido-4-t-butylphenol	225–6°	$C_{12}H_{16}ClNO_2$	C, H, N
26	2,3-Dihydro-5,7-dimethyl-1,4-benzoxazin-3-one	205–9°	$C_{10}H_{11}NO_2$	C, H
27	2,3-Dihydro-6,7-dimethyl-1,4-benzoxazin-3-one	250–1°	$C_{10}H_{11}NO_2$	C, H, N
40	α-Chloro-2-hydroxy-5-methyl-propionanilide	121–2°	$C_{10}H_{12}ClNO_2$	C, H, N
41	2-(*m*-Trifluoromethylbenzamido)-4-chlorophenol	201–2°	$C_{14}H_9ClF_3NO_2$	C, H, N
43	β-(4-Methyl-2-nitrophenoxy)propionic acid	111–2°	$C_{10}H_{11}NO_5$	C, H, N
44	β-(4-Chloro-2-nitrophenoxy)propionic acid	127–8°	$C_9H_8ClNO_5$	C, H, N
45	2-(3,4-Dichlorobenzamido)phenol	193–5°	$C_{13}H_9Cl_2NO_2$	C, H, N
46	2-(3,4-Dichlorobenzamido)-4-methyl-phenol	230–1°	$C_{14}H_{11}Cl_2NO_2$	C, H, N
48	Ethyl 2-(3,4-dichlorobenzamido)-phenylacetate	124–5°	$C_{17}H_{15}Cl_2NO_3$	C, H, N
49	Ethyl 2-(3,4-dichlorobenzamido)-4-methylphenylacetate	134–5°	$C_{18}H_{17}Cl_2NO_3$	C, H, N
50	Ethyl-4-chloro-2-(*m*-trifluoromethyl-benzamido)phenylacetate	86–8°	$C_{18}H_{15}ClF_3NO_3$	C, H, N
56	2-Amino-4-chlorocinnamic acid	209–10°	$C_9H_8ClNO_2$	C, H

The respective phenols required for the synthesis of compounds 26, 27 and 33 were not isolated. The phenol required for compound 47 was the same as used for compound 41.

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