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The effects of substituting tetrazole for carboxyl in two series of anti-inflammatory phenoxyacetic acids

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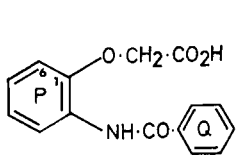
Series of *o*-phenylcarbamoyl- and *o*-benzamido-phenoxymethyl tetrazoles and *o*-phenylcarbamoylphenoxyacetic acids have been synthesized. Anti-inflammatory activity was measured by the phenyl benzoquinone writhing test in mice and the rat foot carrageenan oedema test. Potency in the two *o*-benzamido substituted series could not be related with structure in a satisfactory manner. Introduction of substituents into the benzene rings of the *o*-phenylcarbamoyl substitued series led to complex changes. When the phenoxy ring was unsubstituted, introduction of *meta*- and *para*-substituents possessing high +ve π constants into the *o*-phenylcarbamoyl ring led to increased potency, and each tetrazole was appreciably more potent than the corresponding acid. When the *o*-phenylcarbamoyl ring was unsubstituted *meta*- and *para*-substituents with high +ve π constants introduced into the phenoxy ring caused increases in potency in the acid series but not in the tetrazole series, and each acid was more potent than the corresponding tetrazole. The two tetrazoles found to be the most active in the mouse writhing test 5-[2-(3,4-dichlorophenylcarbamoyl)phenoxymethyl]tetrazole (compound 12T, SNR.2337) and 5-[4-chloro-2-(3-trifluoromethylphenylcarbamoyl)phenoxymethyl]tetrazole (compound 22T, SNR.2420) were selected for study in a series of other anti-inflammatory tests.

The preparation and anti-inflammatory activity of a series of phenyl- and phenoxyalkanoic acids have been described previously (Drain, Daly & others, 1970). In addition to modification of the acidic side chain other structural variations were considered which might lead to compounds with higher activity.

The tetrazole group has an acidic hydrogen which is known to compare with the carboxyl group in pK_a (Mihina & Herbst, 1950; McManus & Herbst, 1959), and as a result several workers have examined tetrazole analogues of physiologically active carboxylic acids in a variety of suitable test systems. Straaten, Solinger & others (1958) found that 5-(4-aminophenyl)tetrazole, the analogue of *p*-aminobenzoic acid, was inactive against *Staphylococcus aureus* and other micro-organisms and the tetrazole analogues of *p*-aminosalicylic acid and isonicotonic acid were inactive against *Mycobacterium tuberculosis*. However, the tetrazole analogue of nicotinic acid assayed as a growth factor substitute for *Lactobacillus arabinosus* showed some activity. McManus & Herbst (1959) prepared a series of tetrazole analogues of amino-acids which were tested (Zygmunt, 1962) as inhibitors of bacterial growth. The compounds were either inactive or very weak growth inhibitors. More recently, Juby, Hudyma & Brown (1968) synthesized a series of 5-(2-anilinophenyl)tetrazoles as analogues of flufenamic acid. They found that the anti-inflammatory activity of each tetrazole was very similar to its corresponding acid, indicating that the replacement of the carboxyl group by tetrazole could lead to active anti-inflammatory agents.

Table 1. *2-Benzamidophenoxyacetic acids and 5-(2-benzamidophenoxymethyl)tetrazoles.*



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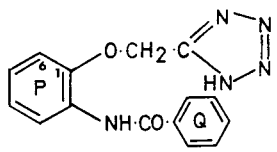

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Cpd. No.	Subt. Ring P	Subt. Ring Q	Method No.	Yield (%)	m.p.[1] °C	Recrystn. solvent	Formula[2]	LD50 mg/kg (oral)	P.B.Q. test (oral)* (mg/kg)	(μmol/kg)	RFT† (oral)[3]
1A	H	H	1A[4]					>1000	140	520	−
1T	H	H	2	64	180–1	Me_2CO–H_2O	$C_{15}H_{13}N_5O_2$	>1000	53	180	+
2A	H	4-Me	1B[4]					>1000	40	140	−
2T	H	4-Me	2	72	194–5	DMF–H_2O	$C_{16}H_{15}N_5O_2$	>1000	80	260	−
3A	H	3,4-Cl_2	1B					>1000	31	91	+
3T	H	3,4-Cl_2	2	90	203–4	Me_2CO–H_2O	$C_{15}H_{11}Cl_2N_5O_2$	>1000	21	58	+
4A	4-Cl	4-Cl	1B					700	150	440	±
4T	4-Cl	4-Cl	2	80	231–2	DMF–H_2O	$C_{15}H_{11}Cl_2N_5O_2$	>1000	22	61	+
5A	4-Cl	3,4-Cl_2	1B					>1000	29	78	+
5T	4-Cl	3,4-Cl_2	2	75	223–4	DMF–H_2O	$C_{15}H_{10}Cl_3N_5O_2$	>1000	17	43	+
6A	4-Me	4-Cl	1B					750	11	35	−
6T	4-Me	4-Cl	2	75	184–5	DMF–H_2O	$C_{16}H_{14}ClN_5O_2$	>1000	30	87	−
7A	4-Me	3,4-Cl_2	1B					1000	44	120	+
7T	4-Me	3,4-Cl_2	2	75	202–3	DMF–H_2O	$C_{16}H_{13}Cl_2N_5O_2$	>1000	42	110	+
8A	4-Me	3-CF_3	1B					750	27	77	−
8T	4-Me	3-CF_3	2	78	186–7	Me_2CO–H_2O	$C_{17}H_{14}F_3N_5O_2$	>1000	10	27	+
9A	5-OMe	4-Cl	1B					>1000	67	200	−
9T	5-OMe	4-Cl	2	90	237–8	DMF–H_2O	$C_{16}H_{14}ClN_5O_3$	>1000	90	250	−
10A	5-OMe	3,4-Cl_2	1B					1000	77	210	−
10T	5-OMe	3,4-Cl_2	2	90	219–20	DMF–H_2O	$C_{16}H_{13}Cl_2N_5O_2$	>1000	90	230	−

* 50% reduction in writhing rate dose.
† Rat foot test (activity at 50 mg/kg).
[1] Melting points are uncorrected.
[2] All compounds were analysed for C,H,N and analytical results obtained for these elements were within ±0·4% of the theoretical values.
[3] *P* values for *t*-tests on rat foot oedema activity are as follows: + = $P < 0{\cdot}05$, ± = $P < 0{\cdot}1$ to $> 0{\cdot}05$, − = $P > 0{\cdot}1$.
[4] Acids prepared by methods 1A and 1B according to Drain & others (1970).

The 2-benzamidophenoxyacetic acids have previously been described in detail (Drain & others, 1970) and are included here for purposes of comparison. In an extension of this work the 2-phenylcarbamoylphenoxyacetic acids have been prepared together with the tetrazole analogues of both acid series.

The methods for assessing anti-inflammatory activity were the PBQ-induced mouse writhing test and the carrageenan rat foot oedema test.

EXPERIMENTAL CHEMISTRY

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

Melting points were recorded on a Gallenkamp manual melting point apparatus.

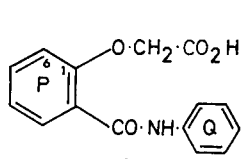
Methods 1A and 1B

For details of these methods of preparation see footnote (4) under Table 1.

Method 2

5-[2-(3,4-*Dichlorobenzamido*)*phenoxymethyl*]*tetrazole* (*cpd no. 3T*). 2-(3,4-*Dichlorobenzamido*)*phenoxyacetonitrile.* To a solution of 2-(3,4-dichlorobenzamido)phenol (5·64 g; 0·02, mol) and chloroacetonitrile (1·89 g; 0·025 mol) in Me_2CO (40 ml) was

Table 2. *2-Phenylcarbamoylphenoxyacetic acids and 5-(2-phenylcarbamoylphenoxymethyl)tetrazoles.*



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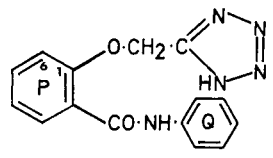

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Cpd. No.	Subt. Ring P	Subt. Ring Q	Method No.	Yield (%)	m.p.[1] °C	Recrystn. solvent	Formula[2]	LD50 mg/kg (oral)	P.B.Q. test (oral)* (mg/kg)	P.B.Q. test (oral)* (μmol/kg)	RFT† (oral)[3]
11A	H	H	4	89	160–1	Me_2CO–H_2O	$C_{15}H_{13}NO_4$[4]	>1000	60	220	–
11T	H	H	2	80	167–8	DMF–H_2O	$C_{15}H_{13}N_5O_2$	>1000	15	51	+
12A	H	3,4-Cl_2	3	87	261–2	AcOH	$C_{15}H_{11}Cl_2NO_4$	>1000	10	29	+
12T	H	3,4-Cl_2	2	85	199–200	AcOH	$C_{15}H_{11}Cl_2N_5O_2$	800	2·1	5·8	+
13A	H	3,5-Cl_2	4	65	218–9	DMF–H_2O	$C_{15}H_{11}Cl_2NO_4$	500	12	35	+
13T	H	3,5-Cl_2	2	75	220–1	Me_2CO–H_2O	$C_{15}H_{11}Cl_2N_5O_2$	>1000	2·7	7·4	+
14A	H	3-CF_3	3	46	215–7	n-Bu_2O	$C_{16}H_{12}F_3NO_4$	1000	19	56	+
14T	H	3-CF_3	2	70	182–3	Me_2CO–H_2O	$C_{16}H_{12}F_3N_5O_2$	>1000	15	41	+
15A	H	4-OMe	4	80	176–7	Me_2CO–H_2O	$C_{16}H_{15}NO_5$	>1000	100	330	–
15T	H	4-OMe	2	80	172–3	Me_2CO–H_2O	$C_{16}H_{15}N_5O_3$	600	13	40	–
16A	4-Cl	H	3	90	202–3	Me_2CO–H_2O	$C_{15}H_{12}ClNO_4$	>1000	7·5	25	+
16T	4-Cl	H	2	70	188–9	Me_2CO–H_2O	$C_{15}H_{12}ClN_5O_2$	1000	23	70	–
17A	4-Br	H	4	60	206–7	n-BuOH	$C_{15}H_{12}BrNO_4$	>1000	3·0	8·6	+
17T	4-Br	H	2	77	176–7	AcOH–H_2O	$C_{15}H_{12}BrN_5O_2$	>1000	20	54	–
18A	4-Me	H	4	86	199–200	Me_2CO–H_2O	$C_{16}H_{15}NO_4$	>1000	4·6	16	+
18T	4-Me	H	2	85	170–1	Me_2CO–H_2O	$C_{16}H_{15}N_5O_2$	750	36	120	–
19A	4-CMe_3	H	4	70	204–5	Me_2CO–H_2O	$C_{19}H_{21}NO_4$	500	6·0	18	+
19T	4-CMe_3	H	2	90	202–3	Me_2CO–H_2O	$C_{19}H_{21}N_5O_2$	>1000	40	110	–
20A	4-Cl	3,4-Cl_2	3	25	260–1	DMF–n-Bu_2O	$C_{15}H_{10}Cl_3NO_4$	>1000	11	29	+
20T	4-Cl	3,4-Cl_2	2	50	230–1	DMF–H_2O	$C_{15}H_{10}Cl_3N_5O_2$	>1000	3·4	8·5	+
21A	4-Cl	4-Me	3	50	217–8	n-Bu_2O	$C_{16}H_{14}ClNO_4$	750	22	69	±
21T	4-Cl	4-Me	2	80	191–2	DMF–H_2O	$C_{16}H_{14}ClN_5O_2$	750	30	87	+
22A	4-Cl	3-CF_3	4	75	214–5	Me_2CO–H_2O	$C_{16}H_{11}ClF_3NO_4$	500	2·0	5·4	+
22T	4-Cl	3-CF_3	2	75	212–3	Me_2CO–H_2O	$C_{16}H_{11}ClF_3N_5O_2$	500	1·4	3·5	+
23A	4-Br	3-CF_3	4	64	189–90	i-PrOH	$C_{16}H_{11}BrF_3NO_4$	>1000	64	150	–
23T	4-Br	3-CF_3	2	73	221–2	AcOH	$C_{16}H_{11}BrF_3N_5O_2$	375	24	54	–
24A	4-Me	4-Cl	4	55	236–7	Me_2CO–H_2O	$C_{16}H_{14}ClNO_4$	>1000	30	94	–
24T	4-Me	4-Cl	2	62	192–3	Me_2CO–H_2O	$C_{16}H_{14}ClN_5O_2$	>1000	14	41	+
25A	4-Me	3,4-Cl_2	3	49	252–3	DMF–n-Bu_2O	$C_{16}H_{13}Cl_2NO_4$	>1000	14	40	+
25T	4-Me	3,4-Cl_2	2	70	212–3	Me_2CO–H_2O	$C_{16}H_{13}Cl_2N_5O_2$	>1000	42	110	+
26A	4-Me	3-CF_3	3	58	225–6	n-Bu_2O	$C_{17}H_{14}F_3NO_4$	1000	13	37	±
26T	4-Me	3-CF_3	2	80	188–9	DMF–H_2O	$C_{17}H_{14}F_3N_5O_2$	1000	14	37	+
27A	4-Et	3,4-Cl_2	4	75	245–7	Me_2CO–H_2O	$C_{17}H_{15}Cl_2NO_4$	375	48	130	+
27T	4-Et	3,4-Cl_2	2	85	212–3	DMF–H_2O	$C_{17}H_{15}Cl_2N_5O_2$	>1000	30	77	–

* 50% reduction in writhing rate dose.
† Rat foot test (activity at 50 mg/kg).
[1] Melting points are uncorrected.
[2] All compounds were analysed for C,H,N and analytical results obtained for these elements were within ±0·4% of the theoretical values.
[3] *P* values for *t*-tests on rat foot oedema activity are as follows: + = $P < 0{\cdot}05$, ± = $P = <0{\cdot}1$ to $>0{\cdot}05$, – = $P > 0{\cdot}1$.
[4] Cohn (1899).

added anhydrous K_2CO_3 (2·27 g; 0·02 mol) and the mixture was boiled under reflux with stirring for 8 h. The cold mixture was poured into aqueous 0·5N NaOH (100 ml) and the precipitate was filtered, washed (H_2O) and dried to afford 6·25 g (97%) of product, m.p. 142–3°. Recrystallization from Me_2CO–H_2O gave the pure product as colourless needles, m.p. 143–4°. Analysis: ($C_{15}H_{10}Cl_2N_2O_2$) C,H,N.

5-[2-(3,4-*Dichlorobenzamido)phenoxymethyl*]*tetrazole.* To a solution of 2-(3,4-dichlorobenzamido)phenoxyacetonitrile (4·01 g; 0·0125 mol) in DMF (30 ml) was added NH_4Cl (0·70 g; 0·0131 mol) and NaN_3 (0·85 g; 0·0131 mol), and the mixture was heated on a steam bath with stirring for 18 h. The solvent was removed *in vacuo* and the resulting oil was dissolved in 1·5N NH_4OH solution (100 ml) and extracted

with EtOAc. The ammoniacal solution was acidified to pH 2 (HCl) and the precipitate was filtered, washed (H_2O) and dried to give 4·48 g (98%) of cpd no. 3T, m.p. 194–5°. Recrystallization from Me_2CO–H_2O afforded 3·88 g (85%) of the pure product as colourless needles, m.p. 203–4°. Analysis: ($C_{15}H_{11}Cl_2N_5O_2$) C,H,N.

Method 3

4-*Methyl*-2-(3-*trifluoromethylphenylcarbamoyl*)*phenoxyacetic acid* (*cpd no.* 26*A*). 4-*Methyl*-2-(3-*trifluoromethylphenylcarbamoyl*)*phenol.* A mixture of 3-trifluoromethylaniline (17·7 g; 0·11 mol) and phenyl 5-methylsalicylate (22·8 g; 0·10 mol) was heated in an oil bath at 200° for 3 h. The product was allowed to cool to 150° and while still fluid was poured into EtOH from which it was recrystallized with charcoal treatment to give 19·2 g (65%) of pure product, m.p. 160–1°. Analysis: ($C_{15}H_{12}F_3NO_2$) C,H.

4-*Methyl*-2-(3-*trifluoromethylphenylcarbamoyl*)*phenoxyacetic acid.* To a solution of Na (1·19 g; 0·052 mol) in EtOH (130 ml) was added 4-methyl-2-(3-trifluoromethylphenylcarbamoyl)phenol (15·26 g; 0·052 mol) with stirring. To this solution was added ethyl chloroacetate (6·32 g; 0·052 mol) and the mixture was boiled under reflux for 7 h. The cool solution was diluted with H_2O (750 ml) and the crude ester was filtered, washed (H_2O) and dried.

The crude ester was dissolved in EtOH (180 ml) containing aqueous N NaOH (53 ml) and boiled under reflux for 7 h. The cold mixture was poured into water (500 ml) containing 5N HCl (15 ml) and the precipitate filtered, washed (H_2O) and dried. Recrystallization from n-Bu_2O afforded 10·7 g (58%) of pure cpd no. 26A as colourless needles, m.p. 225–6°. Analysis: ($C_{17}H_{14}F_3NO_4$) C,H,N.

Method 4

2-(3,4-*Dichlorophenylcarbamoyl*)-4-*ethylphenoxyacetic acid* (*cpd no.* 27*A*). 2-*Carboxy*-4-*ethylphenylacetate.* A solution of 2-carboxy-4-ethyl-phenol (50 g; 0·3 mol) in Ac_2O (150 ml) containing H_2SO_4 (0·1 ml) was heated at 70° for 4 h. The solution was concentrated *in vacuo*, poured into cold H_2O (1·5 litre) and the precipitated solid was ground, washed (H_2O) and dried to afford 59 g of crude 2-carboxy-4-ethylphenylacetate, m.p. 122–5°. Recrystallization from C_6H_6 gave 40 g (64%) of pure product as colourless needles, m.p. 135–7°.

2-(3,4-*Dichlorophenylcarbamoyl*)-4-*ethylphenylacetate.* A suspension of 2-carboxy-4-ethylphenylacetate (20 g; 0·096 mol) in $SOCl_2$ (14·5 ml; 0·2 mol) containing DMF (0·1 ml) was left to stand at room temperature for 16 h and finally was boiled under reflux for 1 h. The excess $SOCl_2$ was removed by co-distillation with several portions of C_6H_6 *in vacuo* and the residual red oil was dissolved in Me_2CO (100 ml). This solution was added during ½ h with stirring to a solution of 3,4-dichloroaniline (16·2 g; 0·1 mol) and Et_3N (12·12 g; 0·12 mol) in Me_2CO (200 ml) and stirred for a further 4 h. The mixture was filtered, the filtrate was concentrated *in vacuo* to 50 ml and poured into 0·1N HCl (500 ml). The resulting oil slowly solidified and was filtered, washed (H_2O) and dried to give 29·5 g (87·5%) of crude product, m.p. 105–10°. Recrystallization from C_6H_6–light petroleum (40–60°) afforded 25·9 g (76·5%) of colourless needles, m.p. 112–4°. A portion recrystallized from C_6H_6 gave m.p. 115–7°. Analysis: ($C_{17}H_{15}Cl_2NO_3$) C,H,N.

2-(3,4-*Dichlorophenylcarbamoyl*)-4-*ethylphenol.* To a stirred suspension of 2-(3,4-dichlorophenylcarbamoyl)-4-ethylphenylacetate (25 g; 0·071 mol) in MeOH (75 ml)

was added a solution of KOH (5·35 g; 0·081 mol) in MeOH (75 ml) during ½ h and the mixture was stirred for 5 h. MeOH (100 ml) was removed *in vacuo* and the resulting oil was poured into 0·1N HCl (1 litre). The precipitate was filtered, washed (H_2O) and dried to afford 21·3 g (97%) of product, m.p. 180–4°. Recrystallization from Me_2CO–H_2O gave 17 g (77%) of the pure product as colourless rods, m.p. 185–7°. Analysis: ($C_{15}H_{13}Cl_2NO_2$) C,H,N.

2-(3,4-*Dichlorophenylcarbamoyl*)-4-*ethylphenoxyacetonitrile.* This compound was prepared by the reaction described under Method 2 to give colourless needles (90%), m.p. 138–40°. Analysis: ($C_{17}H_{14}Cl_2N_2O_2$) C,H,N.

2-(3,4-*Dichlorophenylcarbamoyl*)-4-*ethylphenoxyacetic acid.* To a solution of KOH (1·65 g; 0·025 mol) in MeOH (100 ml) was added 2-(3,4-dichlorophenylcarbamoyl)-4-ethylphenoxyacetonitrile (7·0 g; 0·02 mol) and the mixture was boiled under reflux for 5 h. The MeOH was removed *in vacuo* and the oil was poured into 0·1N HCl (250 ml). The precipitated solid was filtered, washed (H_2O) and dried to afford 7·0 g (95%) of product, m.p. 238–45°. Recrystallization from Me_2CO–H_2O gave 5·52 g (75%) of pure cpd no. 27A as colourless needles, m.p. 245–7°. Analysis: ($C_{17}H_{15}Cl_2NO_4$) C,H,N.

EXPERIMENTAL PHARMACOLOGY

Methods

Acute toxicity. Male albino mice, Smith & Nephew Research (SNR) strain, 25–30 g, 4 animals/dose were given the test compounds by mouth or intraperitoneally.

Table 3. *Intermediates not listed in the literature.*

Intermediate for compound	Compound	m.p.[1] °C	Formula	Analyses[2]
1T	2-Benzamidophenoxyacetonitrile	130–1	$C_{15}H_{12}N_2O_2$	C,H,N
2T	2-(4-Methylbenzamido)phenoxyacetonitrile	129–30	$C_{16}H_{14}N_2O_2$	C,H,N
3T	2-(3,4-Dichlorobenzamido)phenoxyacetonitrile	143–4	$C_{15}H_{10}Cl_2N_2O_2$	C,H,N
4T	2-(4-Chlorobenzamido)-4-chlorophenoxyacetonitrile	156–7	$C_{15}H_{10}Cl_2N_2O_2$	C,H,N
5T	4-Chloro-2-(3,4-dichlorobenzamido)phenoxyacetonitrile	163–4	$C_{15}H_9Cl_3N_2O_2$	C,H,N
6T	2-(4-Chlorobenzamido)-4-methylphenoxyacetonitrile	146–7	$C_{16}H_{13}ClN_2O_2$	C,H,N
7T	2-(3,4-Dichlorobenzamido)-4-methylphenoxyacetonitrile	160–1	$C_{16}H_{12}Cl_2N_2O_2$	C,H,N
8T	4-Methyl-2-(3-trifluoromethylbenzamido)phenoxyacetonitrile	145–6	$C_{17}H_{13}F_3N_2O_2$	C,H,N
9T	2-(4-Chlorobenzamido)-5-methoxyphenoxyacetonitrile	152–3	$C_{16}H_{13}ClN_2O_3$	C,H,N
10T	2-(3,4-Dichlorobenzamido)-5-methoxyphenoxyacetonitrile	159–60	$C_{16}H_{12}Cl_2N_2O_3$	C,H,N
11T	2-Phenylcarbamoylphenoxyacetonitrile	155–6	$C_{15}H_{12}N_2O_2$	C,H,N
12T	2-(3,4-Dichlorophenylcarbamoyl)phenoxyacetonitrile	148–9	$C_{15}H_{10}Cl_2N_2O_2$	C,H,N
13T	2-(3,5-Dichlorophenylcarbamoyl)phenoxyacetonitrile	179–80	$C_{15}H_{10}Cl_2N_2O_2$	C,H,N
14A	2-(3-Trifluoromethylphenylcarbamoyl)phenol	184–6	$C_{14}H_{10}F_3NO_2$	C,H
14T	2-(3-Trifluoromethylphenylcarbamoyl)phenoxyacetonitrile	131–2	$C_{16}H_{11}F_3N_2O_2$	C,H,N
15T	2-(4-Methoxyphenylcarbamoyl)phenoxyacetonitrile	100–1	$C_{16}H_{14}N_2O_3$	C,H,N
16T	4-Chloro-2-phenylcarbamoylphenoxyacetonitrile	116–7	$C_{15}H_{11}ClN_2O_2$	C,H,N
17T	4-Bromo-2-phenylcarbamoylphenoxyacetonitrile	132–3	$C_{15}H_{11}BrN_2O_2$	C,H,N
18T	4-Methyl-2-phenylcarbamoylphenoxyacetonitrile	182–3	$C_{16}H_{14}N_2O_2$	C,H,N
19A	4-t-Butyl-2-phenylcarbamoylphenylacetate	170–2	$C_{19}H_{21}NO_3$	C,H,N
	4-t-Butyl-2-phenylcarbamoylphenol	169–70	$C_{17}H_{19}NO_2$	C,H,N
19T	4-t-Butyl-2-phenylcarbamoylphenoxyacetonitrile	128–30	$C_{19}H_{20}N_2O_2$	C,H,N
20T	4-Chloro-2-(3,4-dichlorophenylcarbamoyl)phenoxyacetonitrile	153–4	$C_{15}H_9Cl_3N_2O_2$	C,H,N
21T	4-Chloro-2-(4-methylphenylcarbamoyl)phenoxyacetonitrile	142–3	$C_{16}H_{13}ClN_2O_2$	C,H,N
22A	4-Chloro-2-(3-trifluoromethylphenylcarbamoyl)phenol	195–6	$C_{14}H_9ClF_3NO_2$	C,H,N
22T	4-Chloro-2-(3-trifluoromethylphenylcarbamoyl)phenoxyacetonitrile	127–8	$C_{16}H_{10}ClF_3N_2O_2$	C,H,N
23A	4-Bromo-2-(3-trifluoromethylphenylcarbamoyl)phenol	205–6	$C_{14}H_9BrF_3NO_2$	C,H,N
23T	4-Bromo-2-(3-trifluoromethylphenylcarbamoyl)phenoxyacetonitrile	142–3	$C_{16}H_{10}BrF_3N_2O_2$	C,H,N
24T	2-(4-Chlorophenylcarbamoyl)-4-methylphenoxyacetonitrile	133–4	$C_{16}H_{13}ClN_2O_2$	C,H,N
25A	2-(3,4-Dichlorophenylcarbamoyl)-4-methylphenol	212–3	$C_{14}H_{11}Cl_2NO_2$	C,H
25T	2-(3,4-Dichlorophenylcarbamoyl)-4-methylphenoxyacetonitrile	141–2	$C_{16}H_{12}Cl_2N_2O_2$	C,H,N
26A	4-Methyl-2-(3-trifluoromethylphenylcarbamoyl)phenol	160–1	$C_{15}H_{12}F_3NO_2$	C,H
26T	4-Methyl-2-(3-trifluoromethylphenylcarbamoyl)phenoxyacetonitrile	129–30	$C_{17}H_{13}F_3N_2O_2$	C,H,N
27A	2-Carboxy-4-ethylphenylacetate	135–7	$C_{11}H_{12}O_4$	—
	2-(3,4-Dichlorophenylcarbamoyl)-4-ethylphenylacetate	115–7	$C_{17}H_{15}Cl_2NO_3$	C,H,N
	2-(3,4-Dichlorophenylcarbamoyl)-4-ethylphenol	185–7	$C_{15}H_{13}Cl_2NO_2$	C,H,N
27T	2-(3,4-Dichlorophenylcarbamoyl)-4-ethylphenoxyacetonitrile	138–40	$C_{17}H_{14}Cl_2N_2O_2$	C,H,N

[1] Melting points are uncorrected.
[2] All analytical results obtained for these elements were within ±0·4% of the theoretical values.

Approximate LD50 values were determined by inspection from mortalities occurring within 3 days.

PBQ writhing test. Female albino mice, SNR strain, four to six weeks old were injected with PBQ 35 min after the oral administration of the test compound. The mice were observed during the 5 min at which maximal writhing occurred in control animals. The number of writhes/mouse were counted and the dose which reduced the writhing rate by 50% was calculated from the dose response curves (10 mice/group) (Litchfield & Wilcoxon, 1949).

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. A suspension of carrageenan (0·1 ml of 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 or 10% gum acacia (controls) had been administered orally. Three h later the foot volume was measured again and the volume of the oedema determined. Results were calculated as percentage inhibition related to the control oedema volume. The oedema volume in control and treated animals was compared using students *t*-test.

Statistics. The PBQ 50% doses in μmol/kg were ranked in ascending order and each result also allotted to one of two groups A or B according to whether the corresponding rat foot result for the compound was positive or negative. The Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956) was used to estimate the probability that groups A and B were from the same population.

Compounds no. 12T (SNR.2337) and 22T (SNR.2420) were selected for further evaluation by the cotton wool pellet granuloma test (Winter & Porter, 1957) fever induction (Brownlee, 1939), tail pinch analgesic (Bianchi & Franchescini, 1954) and ultraviolet erythema test (Winder, Wax & others, 1958).

RESULTS AND DISCUSSION

The degree of activity in the PBQ test varied from the low level of aspirin (110 mg/kg) and phenylbutazone (100 mg/kg) as shown by compounds 1A, 4A and 15A to the most potent derivatives (compounds 12T, 13T and 22T) which were approximately 50 times more active. The most potent of these (compound 22T) had the same order of activity in the writhing test as indomethacin. Although active anti-inflammatory agents consistently produce significant reductions in rat foot volume, the absolute values for the percentage reductions vary from day to day. For this reason the results in this test have been expressed as + (active) or − (inactive) corresponding to significant levels of $P = <0{\cdot}05$ and $>0{\cdot}1$ respectively.

Kruskal–Wallis one-way analysis of variance by ranks gave a value of $P = 0{\cdot}001$ indicating that the rat foot test +ve group ranked by the PBQ results did not come from the same population as the rat foot test −ve group similarly ranked. This evidence in addition to that discussed in a previous paper supports the assumption that in this series the PBQ results indicate anti-inflammatory activity, and results in the latter tests were used to guide the synthetic program.

Correlations between structure and activity for the series of acids in Table 1 were discussed previously. Correlations in the other series and the differences in activity between acid-tetrazole pairs are discussed with reference to ring P and ring Q.

Series in Table 1

Little correlation between structure and activity was apparent within the ten pairs

of compounds in this series. Insufficient compounds are presented to illustrate a preferred substituent for either ring P or ring Q.

Series in Table 2

The introduction of substituents into ring Q with ring P unsubstituted increased activity in the PBQ test in both series beyond that of the unsubstituted compounds (11A and 11T), the increase depending largely on the lipophilic character of the substituent group. Maximum activity corresponded with maximum lipid solubility as expressed by the Hansch substituent constant, π (Hansch & Fujita, 1964) for both acids and tetrazoles (compounds 12A and 12T, 13A and 13T). In this section all five tetrazoles were more active than the corresponding acids, the most active tetrazole being five times more active than the corresponding acid on a molar basis. Many non-steriodal anti-inflammatory compounds contain a carboxylic acid function and the tetrazole analogues of some of these have been synthesized. These tetrazole derivatives tend to be inactive or less active than the corresponding acids. It appears that this sub-series of five pairs of compounds represents the first instance of tetrazole analogues which have a higher activity in a biological test than the corresponding acids.

The introduction of substituents into ring P with no substituent in ring Q caused increases in activity in the acid series but not in the tetrazole series. The introduction of substituents into both rings led to more complicated effects not explained simply in terms of the different π constants.

The most active tetrazoles were compounds 12T, 13T, 20T and 22T with PBQ writhing figures of 5·8, 7·4, 8·5 and 3·5 μmol/kg respectively and the most active acids in this test, compounds 17A and 22A, gave figures of 8·6 and 5·4 μmol/kg. All of these compounds were from Table 2. Several of these more active compounds were examined by other anti-inflammatory procedures and compounds 12T (SNR.2337) and 22T (SNR.2420) were selected for further study.

Table 4 gives results of these further tests.

Table 4. *Results obtained with compounds 12T (SNR.2337) and 22T (SNR.2420) in five anti-inflammatory tests and one analgesic test, in comparison with indomethacin and phenylbutazone.*

Test	Compound 12T (SNR.2337)	Compound 22T (SNR.2420)	Indomethacin	Phenylbutazone
PBQ (50% dose mg/kg oral) ..	2·1	1·4	1·3	100
Rat foot oedema (threshold dose mg/kg oral)	10	8	<1·0	10
Antipyresis in rats[1] (temperature index[2] at 30 mg/kg, oral) ..	1·0	1·0	—	0·62
Mouse tail pinch analgesic test[3]	−ve at 320 mg/kg oral	−ve at 200 mg/kg oral	—	—
Cotton wool pellet[4] granuloma test (threshold dose mg/kg oral)	12	12	0·33	10
Guinea-pig, erythema test (threshold dose mg/kg oral) ..	40–80	40–80	—	20

[1] Brownlee (1939).
[2] Winter, Risley & Nuss (1963).
[3] Bianchi & Franchescini (1954).
[4] Winter & Porter (1957).
[5] Winder & others (1958).

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