JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1505–1514

Received February 15, 2001
Accepted July 16, 2001
ISSN 0022-3573

Design, synthesis and biological testing of a novel series of anti-inflammatory drugs

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Abstract

Many of the non-steroidal anti-inflammatory drugs (NSAIDs) currently marketed produce severe gastro-toxic side effects. The benefits of producing NSAIDs without these side effects are obvious, particularly for patients requiring long-term therapy. The aim of this investigation was to produce novel NSAIDs, based on paracetamol, that exhibit little or no gastro-toxicity. The work covers design, synthesis and testing of 13 drug candidates. The analgesic and anti-inflammatory potencies of the drug candidates were measured using the mouse abdominal constriction assay and the carrageenan-induced rat paw oedema assay, respectively. The stomachs of the rats were examined post-mortem, to assess the gastro-toxicity of the drugs. Of the 13 compounds described herein, 11 were shown to possess analgesic activity at 2–10 times the potency of aspirin, while 8 demonstrated anti-inflammatory activity at 3–10 times the potency of aspirin. Significantly, all of the compounds showed very low gastro-toxicity when compared with aspirin. The results of this study indicate that it is possible to develop novel, potent NSAIDs based on the structure of paracetamol. These compounds have the advantage of demonstrating much lower gastro-toxicity than NSAIDs currently available. Drugs of this type may, in future, provide effective treatments for inflammatory disorders.

Introduction

Non-steroidal anti-inflammatory analgesic drugs (NSAIDs) are used to alleviate mild to moderate pain. They are also used for the treatment of inflammatory disorders such as arthritis and spondylitis. Although there are more than 20 drugs of this type regularly prescribed in the UK, there are several reasons why the production of new drugs is desirable.

Patients show considerable variation in their response to NSAID therapy. Only 60% of patients will respond to any particular NSAID (Mehta 1997) and in the treatment of specific disease states one drug may be more effective than another. Thus, it is to the advantage of the physician to have a wide range of drugs from which to choose. Another consideration is the incidence of gastro-intestinal side effects, termed NSAID gastropathy, which result from treatment with standard NSAIDs. These side effects include discomfort, nausea, diarrhoea, vomiting, petechial lesion and ulcer formation. Gastro-intestinal intolerance is the main reason for cessation of anti-inflammatory therapy. Apart from the distress suffered by the patient, the cost of treating NSAID-induced side effects amounts to billions of dollars annually (Wallace & Granger 1992). For these reasons, this study was undertaken to design novel NSAIDs that would show little or no gastro-intestinal toxicity.

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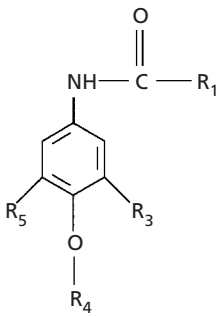
Table 1 Structures of compounds synthesised.



Compound	R1	R3	R5	R4
1	n-C_4H_9	H	H	H
2	CH_3	H	H	n-C_4H_9
3	n-C_4H_9	H	H	n-C_4H_9
4	CH_3	CH_3	CH_3	C_2H_5
5	CH_3	C_2H_5	C_2H_5	C_2H_5
6	CH_3	iPr	iPr	C_2H_5
7	CH_3	Cl	Cl	C_2H_5
8	C_6H_6	H	H	CH_3
9	CH_3	C_2H_5	C_2H_5	CH_3
10	CH_3	CH_3	CH_3	Benzyl
11	CH_3	Benzyl	H	H
12	CH_3	C_6H_6	C_6H_6	H
13	CH_3	H	H	^{t}Bu
14	CH_3	^{t}Bu	^{t}Bu	H

Previous research by Tomlinson (1971) showed that a parabolic relationship existed between the analgesic activity of substituted *N*-phenylacetamides and their 1-octanol/water partition coefficients (log P), for compounds where log P was less than 1.7. Continuing this work, O'Hara (1976) showed that a parabolic relationship existed between analgesic activity and log P of ring-substituted alkyl derivatives of paracetamol, where log P was less than 1.8. Dearden et al (1980) later extended this range of compounds to include derivatives in which the log P was increased to 4.4. In this extended series, a second parabolic relationship was apparent in the log P range 1.8–4.4. This unexpected second parabola led to speculation that the drugs with the higher log P values were acting to produce an anti-inflammatory effect. The analgesic activity had been measured using the mouse abdominal constriction assay, for which there is evidence of an inflammatory component (Collier et al 1968). Since many commercially available NSAIDs have log P values within this range, it was considered possible that a paracetamol derivative with potent analgesic activity could be synthesised which would also possess anti-inflammatory activity.

Paracetamol itself was selected as the parent structure for all of the drugs in this study. The potential use of paracetamol derivatives presented an interesting opportunity for drug development. Paracetamol is not an anti-inflammatory drug, but its derivatives have been found here to possess anti-inflammatory activity. Paracetamol does not exhibit gastro-toxicity, and this favourable characteristic has been maintained in the drugs of this study. This represents an advantage over currently available anti-inflammatory drugs. In this study a maximum of three new drugs was synthesised and tested simultaneously. This ensured that any alteration in structure, which led to an increase or a decrease in activity, could readily be identified. This structural modification could then be incorporated in, or excluded from, the next series of drugs as appropriate. This iterative process allowed for rapid structural optimisation, with the minimum number of drugs requiring synthesis and testing. The structures of the compounds synthesised are shown in Table 1.

Materials and Methods

Materials

4-Amino-2,6-dichlorophenol, 4-aminophenol, benzyl bromide, 2,6-diphenylphenol, 2,6-diethylaniline, iodoethane, 4-methoxyaniline, *N*-(4-methyl phenyl) acetamide and pentanoic anhydride were obtained from Aldrich Chemical Company. Carrageenan was supplied by Bhanda Chemicals and Lambda Pharmaceuticals. Benzyl chloride, tertiary butyl bromide, calcium chloride, ethanoic anhydride, hydrochloric acid, nitric acid, petroleum ether (boiling range 30–60°C), silica gel, sodium carbonate, sodium bicarbonate, sodium dithionite, metallic sodium, sodium nitrite, sodium sulfate, sulfanilic acid, sulfuric acid and granulated tin were obtained from BDH Ltd. *N*-(3,5-ditertiary butyl-4-hydroxyphenyl) acetamide, *N*-(3,5-diethyl-4-hydroxyphenyl) acetamide, *N*-(3,5-diisopropyl-4-hydroxyphenyl acetamide and *N*-(3,5-dimethyl-4-hydroxyphenyl) acetamide were available in our laboratory, synthesised previously by O'Hara (1976).

Chemical synthesis

Compounds were characterised using infra-red spectroscopy (Pye-Unicam 100 SP3). Liquids were analysed as films, solids as nujol mulls; air was used as reference beam. Melting points were determined using Gallenkamp melting point apparatus, and are uncor-

rected; literature values are quoted where available. Molecular masses were obtained using electrospray mass spectrometry (Micromass VG Platform II instrument, MassLynx software, positive ion mode).

N-*(4-hydroxyphenyl)pentanamide (Compound **1**)*
Pentanoic anhydride (0.04 mol) was added to a suspension of 4-aminophenol (0.04 mol) in 12 mL distilled water. The mixture was heated on water-bath for 10 min. After cooling, the precipitate was filtered through a Buchner funnel and dried. Traces of pentanoic acid were removed by stirring with 100 mL of 1.2 M sodium bicarbonate for 20 min. The mixture was filtered and the product was recrystallised from water.

Yield 3.5 g; mp 99.5°C; IR (cm^{-1}) 1650 (C═O amide); measured mass 193.27; calculated mass 193.25.

N-*(4-butoxyphenyl)acetamide (**2**)*
The method was adapted from that of Cizmarik et al (1976).

Metallic sodium (0.1 mol) was dissolved in 40 mL anhydrous ethanol under reflux with a calcium chloride closure. 4-Acetamidophenol (0.1 mol) suspended in 10 mL anhydrous ethanol was added, followed by 0.1 mol 1-chlorobutane. The resulting solution was stirred magnetically for 18 h at room temperature, then heated under reflux for 3 h. The mixture was filtered to remove sodium chloride precipitate. Ethanol was removed by rotary evaporation. Cold distilled water (200 mL) was added to the residue. The crude product was washed with 2.5 M sodium hydroxide (50 mL), then with distilled water until washings were neutral. The product was recrystallised from distilled water.

Yield 11.4 g; mp 109.4°C (literature mp 109°C (Cizmarik et al (1976)); IR (cm^{-1}) 1240 and 1260 (aralkylether); measured mass 207.33; calculated mass 207.27.

N-*(4-butoxyphenyl)pentanamide (**3**)*
Metallic sodium (0.03 mol) was dissolved in 12 mL anhydrous ethanol as above. Compound **1** (0.03 mol, prepared as above) suspended in 3 mL anhydrous ethanol was added, followed by 0.03 mol 1-chlorobutane. The procedure continued as for the synthesis of compound **2**, and the product was recrystallised from 50% ethanol.

Yield 1.9 g; mp 87.1°C; IR (cm^{-1}) 1250 and 1290 (aralkylether); measured mass 249.46; calculated mass 249.35.

N-*(3,5-dimethyl-4-ethoxyphenyl)acetamide (**4**)*
Metallic sodium (0.01 mol) was dissolved in 4 mL anhydrous ethanol as above. A suspension of 0.01 mol *N*-(3,5-dimethyl-4-hydroxyphenyl)acetamide in 3 mL anhydrous ethanol was added, followed by 0.01 mol iodoethane. After stirring for 18 h and heating for 3 h in darkness, the sodium iodide precipitate was removed by filtration. Ethanol was removed by rotary evaporation and the crude product was washed with 10 mL of 1.25 M sodium hydroxide solution, then distilled water until washings were neutral. The product was recrystallised from 50% ethanol.

Yield 1.6 g; mp 133.5°C (literature mp 132–133°C (Noguchi et al (1984)). IR (cm^{-1}) 1210 and 1280 (aralkylether); measured mass 207.34; calculated mass 207.27.

N-*(3,5-diethyl-4-ethoxyphenyl)acetamide (**5**)*
The procedure for synthesis of compound **4** was followed using 0.01 mol *N*-(3,5-diethyl-4-hydroxyphenyl)acetamide in place of dimethyl derivative.

Yield 1.15 g; mp 96°C; IR (cm^{-1}) 1200 and 1270 (aralkylether); measured mass 235.45; calculated mass 235.32.

N-*(3,5-di-isopropyl-4-ethoxyphenyl)acetamide (**6**)*
The procedure for synthesis of compound **4** was followed using 0.01 mol *N*-(3,5-di-isopropyl-4-hydroxyphenyl)-acetamide in place of dimethyl derivative.

Recrystallisation from 50% aqueous ethanol produced impure product. The product was recrystallised, with difficulty, from distilled water, hence only sufficient was recrystallised to perform animal testing.

Yield – not determined; mp 112°C; IR (cm^{-1}) 1260 (aralkylether); measured mass 263.48; calculated mass 263.38.

N-*(3,5-dichloro-4-ethoxyphenyl)acetamide (**7**)*
Part 1. Synthesis of N-(3,5-dichloro-4-hydroxyphenyl)-acetamide. The procedure for synthesis of compound **1** was followed, using 0.05 mol 4-amino-2,6-dichlorophenol suspended in 24 mL distilled water and adding 0.1 mol ethanoic anhydride.
Yield 5.7 g; IR (cm^{-1}) 1660 (C═O amide).

Part 2. Synthesis of N-*(3,5-dichloro-4-ethoxyphenyl)-acetamide*. Metallic sodium (0.026 mol) was dissolved in 12 mL anhydrous ethanol as above. A suspension of 0.026 mol of *N*-(3,5-dichloro-4-hydroxyphenyl)aceta-

mide in 6 mL ethanol was added. The procedure continued as for synthesis of compound **4**.
Yield 3.2 g; mp 173°C; IR (cm^{-1}) showed phenolic OH peak at 2750–3380 diminished; measured mass 247.29; calculated mass 247.14.

N-*(4-methoxyphenyl)benzamide (**8**)*
4-Methoxyaniline (0.05 mol) was suspended in 45 mL 2.5 M sodium hydroxide. Benzoyl chloride (0.05 mol) was added slowly, the mixture was heated on a water-bath for 10 min and then cooled. The precipitate was filtered and impurities were removed by mixing product with warm water, filtering and drying.

Yield 7.8 g; mp 159°C; IR (cm^{-1}) 1640 (C═O amide); measured mass 227.35; calculated mass 227.26.

N-*(3,5-diethyl-4-methoxyphenyl)acetamide (**9**)*
Part 1. Synthesis of 2,6-diethylphenol. Concentrated sulfuric acid (3 mol) was added to 1200 mL distilled water. 2,6-Diethylaniline (1.5 mol) was added and the mixture was heated on a steam bath for 15 min, followed by cooling in ice-water to 5°C. Sodium nitrite (1.5 mol) in 200 mL water was slowly added, and once the reaction had subsided, the mixture was heated to 50°C on a steam bath for 1 h. After standing overnight the resultant precipitate was filtered and dried. Sample was recrystallised from 50% aqueous ethanol.

IR (cm^{-1}) 3200–3600 (phenolic OH).

Part 2. Synthesis of 2,6-diethylanisole. 2,6-Diethylphenol (1 mol) was dissolved in chloroform and dried using anhydrous sodium sulfate. Chloroform was removed in a rotary evaporator. Metallic sodium (0.27 mol) was dissolved in 108 mL anhydrous ethanol as above. Dried 2,6-diethylphenol (0.67 mol) was added to the sodium ethylate, followed by 0.27 mol iodomethane, and synthesis continued as for product **2**. Distilled water (50 mL) was added to remove water-soluble impurities, and the product was extracted into 75 mL chloroform, which was subsequently removed by rotary evaporation.

IR (cm^{-1}) 1200 and 1260 (aralkylether), also disappearance of phenolic OH peak at 3200–3600.

Part 3. Synthesis of 2,6-diethyl-4-nitroanisole. The method was adapted from that of Bisarbi-Ershadi & Rigterink (1989).

2,6-Diethylanisole (0.66 mol) was added to 660 mL chloroform in a 3-necked round-bottomed flask fitted with thermometer, dropping funnel and magnetic stirrer. Nitric acid (0.5 mol, 70%) was added over 1.5 h with stirring and cooling in ice to 25–30°C. The mixture was left for 5 h, then 220 mL distilled water was added. The organic layer was removed and washed with water until neutral. Chloroform was removed by rotary evaporation and the resultant oil was returned to the reaction vessel. A further 0.5 mol 70% nitric acid was added and the mixture was stirred for 5 h. Distilled water (220 mL) was added and the chloroform layer was separated, washed and chloroform removed by rotary evaporation.

IR (cm^{-1}) 1340 and 1510–1530 (aromatic NO_2).

Part 4. Synthesis of 3,5-diethyl-4-methoxyaniline. 2,6-Diethyl-4-nitroanisole (0.33 mol) was added to 0.5 mol granulated tin in a round-bottomed flask fitted with 2 reflux condensers. Concentrated hydrochloric acid (4.3 mol) was added slowly and the mixture was warmed on a steam bath. After the reaction subsided, the mixture was cooled and 2.5 mol sodium hydroxide in 166 mL water was added slowly. Chloroform (200 mL) and water (200 mL) were added and the mixture was filtered. The product was repeatedly extracted into chloroform until the chloroform layer was colourless. Combined chloroform extractions were washed with 2.5 M sodium hydroxide (100 mL), then water until neutral. Chloroform was removed by rotary evaporation.

IR (cm^{-1}) 1610 (NH_2), also disappearance of NO_2 peaks at 1340 and 1510–1530.

Part 5. Synthesis of N-*(3,5-diethyl-4-methoxyphenyl)-acetamide.* 3,5-Diethyl-4-methoxyaniline (0.18 mol) was suspended in 45 mL distilled water and 0.2 mol ethanoic anhydride was added. The mixture was stirred vigorously, heated for 10 min, then cooled. Sodium bicarbonate (200 mL, 0.6 M) was added and the mixture was stirred for a further 30 min. Chloroform (250 mL) was added. The chloroform layer was separated and washed 3 times with 200 mL distilled water. Chloroform was removed by rotary evaporation, leaving a red oil. This was purified, with difficulty, from 70% aqueous ethanol solution, hence only sufficient was recrystallised to allow for animal testing.

Yield – not determined; mp 94.5–95°C; IR (cm^{-1}) 1660 (C═O amide); measured mass 221.30; calculated mass 221.30.

N-*(3,5-dimethyl-4-benzyloxyphenyl)acetamide (**10**)*
Metallic sodium (0.02 mol) was dissolved in 10 mL anhydrous ethanol as above. A suspension of 0.02 mol *N*-(3,5-dimethyl-4-hydroxyphenyl)acetamide in 10 mL ethanol was added, followed by 0.001 mol ascorbic acid

and 0.02 mol benzyl bromide. The mixture was stirred overnight, heated under reflux for 3 h, then cooled. The product was extracted into 30 mL chloroform. Chloroform was removed by rotary evaporation and the compound was recrystallised from 50% aqueous ethanol.

Yield 2.4 g; mp 148°C; IR (cm^{-1}) absence of phenolic OH peak at 3300 cm^{-1}; measured mass 269.34; calculated mass 269.34.

N-*(3-benzyl-4-hydroxyphenyl)acetamide (**11**)*

Part 1. Synthesis of 3-benzyl-4-aminophenol. The method was adapted from that of O'Hara (1976).

Sulfanilic acid (0.25 mol) and sodium carbonate (0.12 mol) were dissolved with heating and stirring in 250 mL distilled water. Sodium nitrite (0.27 mol) was dissolved in 80 mL distilled water and the two solutions were cooled in ice to 15°C. Both solutions were added to 1.7 mol concentrated hydrochloric acid in 300 g crushed ice, giving mixture A, which was allowed to stand for 20 min.

2-Benzylphenol (0.25 mol) was dissolved in a solution of 1.38 mol sodium hydroxide in 300 mL distilled water and cooled by the addition of 200 g ice. This solution was added to mixture A and allowed to stand for 3 h, after which it was heated to 45°C and 0.6 mol sodium dithionite was added. The mixture was heated to 60–65°C and the temperature was maintained until product appeared. The product was filtered and washed with 0.03 M sodium dithionite solution.

IR (cm^{-1}) 1500 and 1510 (aromatic NH_2), 3300 (phenolic OH).

Part 2. Synthesis of N-*(3-benzyl-4-hydroxyphenyl)-acetamide*. 3-Benzyl-4-aminophenol (0.16 mol) was suspended in 60 mL distilled water. Ethanoic anhydride (0.16 mol) was added and the mixture was heated for 10 min. The resulting solid was filtered and mixed with 1.2 M sodium bicarbonate (200 mL), then filtered and washed with water. The product was recrystallised, with difficulty, from 0.03 M sodium dithionite solution, hence only sufficient was purified to allow for animal testing.

Yield – not determined; mp 128.5–129.5°C. IR (cm^{-1}) 1620–1640 (C═O amide); measured mass 241.30; calculated mass 241.29.

N-*(3,5-diphenyl-4-hydroxyphenyl)acetamide (**12**)*

The procedure for synthesis of compound **11** was followed. 2,6-Diphenylphenol replaced 2-benzylphenol as the starting material and was suspended in a solution of 1.1 mol sodium hydroxide in 300 mL distilled water containing 25 mL ethanol. The product was recrystallised, with difficulty, from 70% ethanol solution, hence only sufficient was purified to allow for animal testing.

Yield – not determined; mp 101–103°C; IR (cm^{-1}) 1620 (C═O amide); measured mass 303.35; calculated mass 303.36.

N-*(4-tertiarybutoxyphenyl)acetamide (**13**)*

The procedure for synthesis of compound **2** was followed. Tertiarybutylbromide was used in place of 1-chlorobutane. Residual 4-acetamidophenol was removed by washing with toluene, which was subsequently removed by rotary evaporation. The product was mixed with 0.6 M sodium bicarbonate (100 mL) for 30 min, washed with distilled water and recrystallised, with difficulty, from 0.12 M sodium bicarbonate in 10% aqueous ethanol. Only sufficient compound was purified to allow for animal testing.

Yield – not determined; mp 126–127°C (literature mp 130°C (Cadogan et al)). IR (cm^{-1}) 1220 and 1240 (aralkylether); measured mass 207.29; calculated mass 207.27.

N-*(3,5-ditertiarybutyl-4-hydroxyphenyl)acetamide (**14**)*

This drug had previously been synthesised by O'Hara (1976).

Pharmacological evaluation

All animal testing was performed in accordance with Home Office regulations and with ethical approval from the University.

Analgesic activity

Analgesic activity was tested using the abdominal constriction assay (Collier et al 1968). Male white CD-1 mice, weighing 25–30 g (bred in-house or obtained from Charles River suppliers), were fasted for 16 h and allowed free access to water. Homogeneous drug suspensions were made in distilled water with 1 drop of Tween 80. Mice were dosed by gavage, each receiving 0.5 mL drug suspension (0.5 mL vehicle for control group).

Thirty minutes post dose, mice were injected intraperitoneally with 0.1 mL of 0.7% v/v acetic acid solution and placed in a deep-sided, open-top plastic box. The number of full abdominal constrictions (comprising a stretching of the hind limbs to full extent allowing the abdomen to touch the cage floor) performed by mice were counted for a 15-min period.

Five mice were used for each of the four dose levels of drug (doses selected from prior knowledge of similar

compounds). A control group of five mice was used on each day of testing, as the test is very susceptible to external conditions. The total number of constrictions was summed for the five mice in each group. Analgesic activity was recorded as the percentage inhibition of abdominal constrictions when drug was present compared with control group. Results were calculated using equation 1

$$\% \text{ Inhibition} = 100 - (100 \times (\text{average drug response}/\text{average control response})) \quad (1)$$

ED50 (the effective dose to inhibit the biological response by 50 %) values were obtained from dose–response plots.

Anti-inflammatory activity

Anti-inflammatory activity was measured using the carrageenan-induced rat paw oedema assay (Winter et al 1962). White male Wistar rats from a breeding stock originally supplied by Charles River were all bred in-house. As facilities allowed for only limited breeding, overall weights ranged from 90–400g. Within individual studies, however, all rats were of comparable weight. Drug suspensions were prepared as described previously. Carrageenan solution (2 %) was prepared using 0.1 g carrageenan from Lambda Pharmaceuticals and 0.1 g carrageenan from Bhanda chemicals in 0.9 % sodium chloride solution (10 mL). Carrageenan solution was prepared 24 h before use and stored in a refrigerator, whereupon it became gelatinous.

Rats were fasted for 16 h before use and allowed free access to water. Rats were dosed by gavage with drug suspension (0.5–2 mL) or vehicle (control group). Thirty minutes post dose each rat was injected with 2 % carrageenan solution (0.1 mL) into the subplantar surface of the right hind paw. A black mark was made at the knee joint for reference. Paw volume, up to the reference mark, was measured plethysmographically immediately following injection and each hour thereafter. Paw volume at the 3-h time point was used for subsequent calculations. Three groups of drug-treated rats and one control group were used each test day. Five rats were used in each group, the mean paw oedema value for the test group being compared with the mean value for the control group for that day:

$$\text{Oedema level} = (\text{paw volume at time t} - \text{volume at time 0})/\text{volume at time 0} \quad (2)$$

Anti-inflammatory activity was measured as the percentage reduction in oedema level when drug was present, relative to control:

Table 2 Scoring system used to determine gastro-toxicity.

Description of lesion	Lesion score
0	0
1–150 minute	1–5
150–200+ minute	5–10
reddened areas	5–10
slight sloughing	5–10
severe sloughing	10–20
1–20 small	10–30
1–5 medium	30–35
5–10+ medium	35–40
1–5 severe	40–50
5–20 severe	50–70
1–10 streak	70–90
10+ streak	90–100

Further details of the scoring system are given in the text.

$$\text{Activity} = 100 - (100 \times (\text{average volume drug treated}/\text{average volume for control})) \quad (3)$$

Four dose levels were used for each drug, from which dose–response graphs were produced. ED30 (the effective dose to inhibit the biological response by 30 %) values were obtained visually from these graphs. ED30 values were used rather than ED50 values so that activities of the less potent compounds could be quantified.

Gastro-toxicity

Gastro-toxicity was measured using a visual lesion assessment method, adapted from Dearden & Nicholson (1984). After paw volumes at 4 h were measured, rats were killed either by injection with 0.25 mL euthatal or by being placed in a CO_2 chamber. Stomachs were removed, rinsed with tap water and stretched over an inverted test-tube. They were examined by eye for signs of damage and scores were recorded for each rat according to the scale shown in Table 2.

Examination of stomachs by eye provided a simple and reliable method to quantify gastric irritancy. Lesions produced by anti-inflammatory drugs were clearly visible and severity could be readily assessed. There are several scales recorded in the literature for estimating gastro-toxicity; however it is difficult to compare results between different groups as the manner of interpreting damage will vary considerably. The ulcerogenic activities of the compounds in this study were therefore assessed relative to each other only and a comparison was made with aspirin, which is commercially available. As the compounds showed very low gastro-toxicity, a scale was devised so that even limited damage could be quantified. As the rats were fasted overnight, they tended

Table 3 Results of the analgesic, anti-inflammatory and gastro-toxicity tests.

Compound	Analgesic ED50 (mol kg^{-1})	Anti-inflammatory ED30 (mol kg^{-1})	Gastro-toxicity score
1	2.4×10^{-4}	Inactive	1.4
2	3.5×10^{-4}	3.6×10^{-4}	1.2
3	2.1×10^{-4}	Inactive	1.7
4	1.9×10^{-4}	1.16×10^{-4}	1.2
5	5.1×10^{-4}	1.7×10^{-4}	1.6
6	4.2×10^{-4}	1.9×10^{-4}	2.3
7	1.1×10^{-4}	3.3×10^{-4}	2.1
8	3.9×10^{-4}	Inactive	1.1
9	3.7×10^{-4}	4.0×10^{-4}	1.8
10	Inactive	Inactive	1.3
11	1.4×10^{-4}	3.3×10^{-4}	1.3
12[a]	Incalculable	Incalculable	1.5
13[b]	49 % inhibition at 2.4×10^{-4}	48 % inhibition at 3.8×10^{-4}	Incalculable
14	4.9×10^{-5}	2.1×10^{-4}	2.6
Aspirin	9.4×10^{-4}	1.1×10^{-3}	9.5

[a]Although analgesic and anti-inflammatory activity was seen the responses were not uniform and therefore estimates of ED50 and ED30 could not be made. [b]Sufficient compound was available to allow testing at only one dose level.

to eat sawdust which could lead to minute lesions on the surface of the stomach. As these were apparent also in the control groups they were not considered to be significant. Consequently, 1 or 2 medium lesions scored much higher than 100 of these minute lesions.

The terms minute, small, medium and severe refer to the diameter of the circular lesions. A streak lesion appears as a furrow along the stomach lining, where a line of lesions has formed, leading to severe breakdown of the mucosa and bleeding into the stomach. Aspirin was the only drug tested that produced this effect.

The average lesion score per rat, for all twenty rats used in producing the anti-inflammatory data, was used irrespective of dose of drug given. These were then scaled to give relative toxicity values (that of control being 1). The scaled gastro-toxicity scores are recorded in Table 3. The incidence of lesions has been shown not to be directly related to the amount of drug given but rather to show a dose–response pattern independent of the dose–response curve for anti-inflammatory activity (Elliott 1979). Therefore, to obtain absolute values for toxicity a separate dose–response study would have to have been undertaken, doubling the number of rats used. The results produced by this study give an indication of the toxicity score obtained at the doses required to give an anti-inflammatory effect. We believe that this gives a most realistic indication of gastro-toxicity.

In-vivo experiments were performed as this gives a better indication of the efficacy and toxicity of the drugs. Experiments performed in-vitro cannot account for pharmacokinetic effects, which may render a drug inactive in-vivo, despite apparent potency in-vitro.

Results and Discussion

The results of the biological tests are shown in Table 3.

Rationale for compound selection

In this study a series of paracetamol derivatives was designed and tested for analgesic and anti-inflammatory activity. The work follows on from the findings of O'Hara (1976) and Dearden et al (1980) vide supra. One important consideration in the design of anti-inflammatory drugs is the problem of gastric irritancy, the most common side effect associated with NSAIDs. As paracetamol is devoid of gastro-toxic effects, the possibility of capitalising on this property, by using paracetamol derivatives, presented the opportunity to improve on current therapies.

The results from O'Hara's work (O'Hara 1976) indicated that substitutions in positions 2 and 6 reduced analgesic activity, whereas substitution in positions 3

and 5 enhanced activity. No attempt had been made by O'Hara to substitute the acetamide or hydroxy functions. In this study, therefore, the first structural alterations were to convert the acetamide function to a pentanamide (compound **1**). The hydroxy group was converted to a butoxy group in compound **2** and both of the modifications were included in compound **3**. The log P values calculated for compounds **1**, **2** and **3** were 2.1, 2.8 and 4.4, respectively, (MedChem software, version 3.52, Medicinal Chemistry Project, Pomona College, USA). These values all fall within the range in which the second parabolic relationship had been demonstrated by Dearden et al (1980). The compounds were therefore predicted to possess anti-inflammatory activity. Although compound **2** (of similar log P to compound **1**) possessed anti-inflammatory activity, compounds **1** and **3** did not. This was attributed to the substitution of the acetamide function with the pentanamide group. In the next iteration of the design cycle therefore the acetamide group was not substituted.

As 3- and 5-substitution had enhanced the activity of O'Hara's compounds, substitutions were made in these positions for compounds **4**, **5** and **6** using methyl, ethyl and isopropyl groups, respectively. We wished to maintain an alkoxy function in place of the hydroxy, as this had proved successful in compound **2**. The butoxy function was not used in position 4 for these compounds, as the log P values would have been too high. An ethoxy group was substituted in position 4 giving calculated log P values of 2.8, 3.8 and 4.6 for compounds **4**, **5** and **6**, respectively. This made synthesis easier as the butoxy group would have created more steric hindrance. The influence of halogen substituents in place of alkyl groups in the 3 and 5 positions was tested in compound **7**. This compound had a calculated log P of 3.1, again falling within the range of the second parabola. Compounds **4**–**7** were found to possess superior efficacy to compound **2**.

To test if a larger group in place of the pentanamide, used in compounds **1** and **3**, would produce an anti-inflammatory effect, compound **8** was synthesised wherein a phenyl amide replaced the acetamide function. Attempts were also made to determine whether an alkoxy or aralkoxy replacement for the hydroxy function in position 4 would improve activity. In compound **8** a methoxy group was used, the calculated log P value being 2.7. Compound **9** represented a modification of compound **5**, possessing a 3,5-diethyl substitution in addition to a methoxy group in position 4 (calculated log P, 3.3). In compound **10** a benzyloxy function was used in position 4 with 3,5 dimethyl substitution (calculated log P 4.0).

As compound **8** showed no anti-inflammatory activity, no further substitutions were made at the acetamide function and as compound **10** was the only compound devoid of both analgesic and anti-inflammatory activity, the use of such a bulky group in position 4 was avoided in the next design–test iteration.

We had carried out a separate study, using the Catalyst Molecular Modelling package, to design an analgesic drug (Duffy et al 1995). That study resulted in the design of compound **11**, which incorporated a bulky, hydrophobic group in position 3. Progressing from compound **11**, compound **12** was designed, in which a bulky group was incorporated in both positions 3 and 5. Calculated log P values were 2.6 and 3.7 for compounds **11** and **12**, respectively.

Compound **13** was designed to test whether replacing the n-butoxy function with a tertiary butoxy function would lead to a significant change in activity. The calculated log P for this compound was 2.5, similar to that of compound **2** (log P, 2.8). Comparing the results for compounds **2** and **13** should distinguish the effects of the topological differences in the substituents in position 4. As there were problems in the synthesis of compound **13**, sufficient could be made only to allow testing at one dosage level.

The calculated log P values for the compounds in this study all fell within the range 2.1–4.6, similar to the values investigated by Dearden et al (1980). However, unlike the previous study, no overall correlation was found between log P and in-vivo analgesic or anti-inflammatory activity. This suggested that factors other than hydrophobicity, such as steric or electronic effects, were more significant in determining activity within this series.

Anti-inflammatory activity

The presence of an acetamide function appeared essential for anti-inflammatory activity. In compounds **1**, **3** and **8**, where the acetamide had been substituted, anti-inflammatory activity was abolished. Replacement of the hydroxy group with a butoxy function enhanced the compound's anti-inflammatory activity. There was little difference in potency between the n-butoxy and the tertiary-butoxy substitutions (compounds **2** and **13**, respectively). In compound **10**, where the benzyloxy function was used, activity was again abolished. The most effective anti-inflammatory agents were those where the hydroxy had been substituted by an ethoxy group (compounds **4**–**7**). Anti-inflammatory potency within this series decreased as the size and hydro-

phobicity of the alkyl substituent increases. This may be due to the optimum log P value having been exceeded or to increasing steric hindrance at the receptor site. Replacement of the alkyl groups in positions 3 and 5 with a chloro group (compound **7**) reduced anti-inflammatory activity, as did replacing the ethoxy in position 4 (compound **5**), with the smaller methoxy group (compound **9**). In compound **11**, substitution was made in position 3, but not in position 5. This substitution pattern also produced an effective drug. Although compound **12** did possess some anti-inflammatory and analgesic activity, the responses obtained were not uniform and therefore could not be quantified. The reason for this is unclear.

Analgesic activity

With the exception of compound **14** (synthesised previously by O'Hara), the di-chloro derivative (compound **7**) was the most effective analgesic. Compound **11**, designed using the Catalyst program, was the second most effective analgesic. Unlike anti-inflammatory activity, analgesic activity was not abolished by substitution of the acetamide function.

All compounds which possessed anti-inflammatory activity possessed analgesic activity, but not vice versa. This may have been because the receptor which mediates analgesic activity is more accommodating than the receptor which mediates anti-inflammatory activity. It may also have been due to the analgesic assay being more susceptible to false-positive results than the anti-inflammatory assay.

The pathways via which pain and inflammation are mediated are particularly complex. Interfering with different mediators of these pathways may result in a more potent analgesic or anti-inflammatory effect. This relationship is also seen in the fenamates, where flufenamic acid is a more potent anti-inflammatory and mefenamic acid is a more potent analgesic (Lembo et al 1983).

For all the compounds where the hydroxy group had been replaced with an alkoxy function, metabolism in the liver may occur via *O*-dealkylation, resulting in the formation of the corresponding substituted phenol (phase I transformation). Ninety percent of paracetamol is metabolised by conversion of the hydroxy to a glucuronide or sulfate conjugate, which is readily excreted in the urine. Theoretically the substituted phenol derivatives, formed via phase I transformation, may also undergo conjugation. However 3,5-disubstitution would hinder access to the hydroxy group, and hence slow metabolism of the drugs. If these compounds do undergo *O*-dealkylation, they would then exist as substituted phenols in the body. Substituted phenols, by acting as free-radical scavengers, have been shown to act as anti-inflammatory agents (Swingle et al 1985). This may account for the activity of these compounds.

The ability of certain drugs to insert into the lipid bilayer of cells which mediate inflammation has also been proposed as a mechanism for anti-inflammatory activity (Hwang & Shen 1981). Once inserted into the membrane, the drugs interrupt cell signalling processes and inhibit activation of inflammatory cells. Paracetamol itself is not sufficiently hydrophobic, but the increased hydrophobicity of the compounds in this study may enable them to act via such a mechanism.

Toxicity

Prostaglandins play a role in mediating inflammation and also in protecting the gastric mucosa. Two major enzyme systems involved in the production of prostaglandins are cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is responsible for maintenance of the gastric mucosa (Wallace & Cirino 1984), with COX-2 responsible for mediating inflammation. Currently available NSAIDs are believed to owe their anti-inflammatory activity and gastro-toxicity to the inhibition of COX-2 and COX-1, respectively. The lack of gastro-toxicity apparent in the drugs synthesised in this study may have been due to their selective inhibition of COX-2, or to the fact that they act by a method other than inhibition of prostaglandin synthesis. Gastro-toxicity of NSAIDs may be caused by a direct irritant effect of the acidic drugs on the gastric mucosa. The lack of gastro-toxicity of these new drugs may be due, in part, to their non-acidic nature. As NSAID gastropathy is the main reason for cessation of anti-inflammatory therapy, these drugs present a possible alternative to those currently available. Although some commercial NSAIDs are more potent, the lack of gastro-toxicity of the drugs described herein may enable higher doses of them to be given more safely.

As these new drugs are based on paracetamol, another important consideration is that of hepatotoxicity. Although no estimation of hepatotoxicity was made in this work, the conclusions drawn by Van de Straat et al (1986) are relevant. Their work on similar substituted paracetamol derivatives showed that although paracetamol and the 3-mono-substituted derivatives did induce hepatotoxicity, 3,5-disubstituted derivatives did not. From these results it is predicted that the 3,5-disubstituted derivatives synthesised in the present work would not be hepatotoxic.

Conclusion

The results show that 11 of the drugs designed in this study possess an analgesic potency 2–10 times that of aspirin, while 8 possess an anti-inflammatory activity 3–10 times that of aspirin. The most significant results, however, relate to the gastro-toxicity profile of these compounds. The toxicity score is below 2.6 for every compound designed. This compares favourably with the value of 9.5 obtained for aspirin. Of all the compounds tested, aspirin was the only drug to produce characteristic streak lesions. Although the drugs synthesised in this study proved potent in the assays used, this is no guarantee of their efficacy in man. Interspecies differences in pharmacokinetics and disease states may render the drugs ineffective in treating human disorders. More detailed pharmacokinetic and toxicity studies on these drugs are essential.

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