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Full Length Research Paper

Reaction of phthalimido alkyl acids with isopropylamine: synthesis, anti-inflammatory and antinociceptive properties

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Phthalimido alkyl acids 3-phthalimidopropionic acid (2a) and 4-phthalimidobutyric acid (2b) were treated with isopropylamine at room temperature using different solvents (dimethylformamide, dichloromethane and methanol) as the reaction medium and afforded 3-benzamido-propionic acid –2-(2-methylethyl)-carboxamide (3), 2-benzamido-2-methylethane-2-(2-methylethyl)-carboxamide (4), and 4-benzamido-butyric acid –2- (2-methylethyl)- carboxamide (5). Compound 4 was a dimmer that was least expected. Compounds 3, 4 and 5 were evaluated for antinociceptive property using acetic acid-induced writhing test. 4 exhibited the highest analgesic effect at 80 mg/kg it caused 88 % inhibition and was more pronounced than the reference drugs (indomethacin, acetylsalicylic acid and paracetamol), 3 and 5. The anti-inflammatory activity of the compounds was also screened using carrageenan- induced rat paw oedema assay and 4 showed higher activity than others except the reference drug. The effects were dose-dependent.

Key words: Phthalimido alkyl acids, mice, antinociception, mouse writhing test, isopropylamine, anti-inflammatory activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) belong to a variety of chemical classes with no common features except the absence of a steroidal structure. Their primary effect is pain relief but also with antipyretic and anti-inflammatory activities. Their pro-algogenic effects are explained by sensitization of nociceptive nerve endings to the stimulating effect (algogenic) of kinins (bradykinin), serotonin and histamine. In addition, production of prostanoid in the brain has a thermoregulatory effect (Devillier, 2001). Prostaglandins sensitize peripheral nerve endings and nociceptors to transmit pain signals to the brain and the spinal cord (Dannhardt and Kiefer, 2001). There are over twenty NSAIDS regularly prescribed in the clinics with undesirable side effects hence the search for newer drugs (Duffy et al., 2001).

The synthesis, analgesic and anti-inflammatory properties of compounds containing free carboxylic acids have been described previously (Vazquez et al., 1996; Nakamura et al., 1983); hence the basis for screening the compounds synthesized for antinociceptive property. The presence of two carbonyl groups in phthalimides increases the acidity of the amino hydrogen, reduces its nucleophilicity of both oxygen and nitrogen atoms, and increases the electron deficiency of carbonyl carbons. Thus nucleophilic addition to the carbonyl carbon atom is frequently encountered. The attack at the carbonyl carbon by nucleophilic reagents such as amine is possible producing ring cleavage (CO – N bond fission) to give diamides; hence the use of isopropylamine to open the phthalimidoalkyl acids.

The aim of this work was to study the effect of isopropylamine on phthalimidoalkyl acids, subsequent opening of the phthalimido moiety and to study the influence of different solvents on the yield, rate of reaction and the product formed. The products formed were then evaluated for anti-inflammatory and antinociceptive properties.

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MATERIALS AND METHODS

Melting points measured were uncorrected. IR spectra were recorded on a Buck scientific IR M500 instrument. NMR spectra were recorded on a Varian Gemini 200. Chemical shifts are reported in ppm relative to tetramethylsilane. Mass spectra were acquired on a Varian MAT 44S mass spectrometer operating at 70eV. Elemental analysis agreed favourably with the calculated values. Analytical thin layer chromatography (TLC) was used to monitor the reactions.

Synthesis of phthalimido alkyl acids (2a-b)

74 g (0.5 mol) of phthalic acid anhydride and 0.5 mol of the respective β-alanine and GABA were refluxed in 300 ml toluene in the presence of 6.5 ml triethylamine for 2 h in a Dean-stark apparatus. The organic solvents were removed *in vacuo*, 70 ml of water and 10 ml of concentrated HCl were added and the mixture stirred for 30 min, filtered and dried. Recrystallization from ethanol yielded 3-phthalimidopropionic acid (**2a**), 99.9 g, 91%, mp 151-152^{o}C (Litt. 150-152 ^{o}C) and 4-phthalimidobutyric acid (**2b**), 95.5 g, 82%, mp116-118^{o}C (Litt. 116-117 ^{o}C).

Synthesis of the carboxamides

3-Benzamido-propionic acid –2-(2-methylethyl) carboxamide (3): To a solution of 3-phthalimidopropionic acid (1.0 g, 4.60 mmol) in 30 ml of methanol : dichloromethane (1:2) was added isopropylamine (0.54 g, 9.20 mmol) and stirred at room temperature for 3 h. The organic solvent was removed *in vacuo*, an oily residue was obtained which slowly crystalised after 5 days to give needle-like crystals (yellowish), and recrystallized from methanol : hexane mixture to give compound **3** (0.74 g, 58.05%), mp 80-82 ^{o}C.

IR (KBr,ν_{max}, cm^{-1}): 3615 (NH), 3412 (OH), 1720 (COOH), 1610 (C=O), 1516 (C-N), 770 (1,2-disubstitution).
^{1}H NMR (200MHz, DMSO-d$_6$, δ): 1.15-1.12 (d, 6H, J = 6.4Hz, -2 X C**H**$_3$), 2.35-2.17 (m, 1H, -C**H**-), 3.26-3.13 (q, 2H, J =6.6Hz -NH-C**H**$_2$-), 3.74-3.66 (t, 2H, J = 6.6Hz, -C**H**$_2$COOH), 5.23 (brs, 1H, -O**H**), 7.52-7.31 (m, 2H, Ar-H), 7.82-7.76 (m, 2H, Ar-H), 8.10 (d, 1H, J = 7.0Hz, N**H**), 8.20 (t, 1H, J = 6.3Hz, N**H**).
^{13}C NMR (50MHz, DMSO-d$_6$, δ): 22.1 (- 2 X CH$_3$), 33.5 (CH$_2$), 34.9 (CH$_2$), 42.3 (-CH), 122.8, 127.6, 128.9, 132.0, 134.2, 135.9 (Ar-C), 167.2 (C=O), 167.7(C=O), 174.6 (COOH).
MS (m/z, 70eV): 278.0 (2%)[M^{+}], 201.9(2), 174.1 (18), 173.0 (100), 160.1 (44), 133.0 (21), 104.1 (38), 76.1 (25), 66.1 (8), 51.0 (9).
Elemental Analysis: Found (%): C, 60.43; H, 6.47; N, 10.07; $C_{14}H_{18}N_2O_4$. Anal. Calcd. (%): C, 60.42; H, 6.52; N, 10.07.

Benzamido - N, N - bis (2 - methylethyl) - carboxamide (4): To a solution of 3-phthalimido propionic acid (1.00 g, 4.56 mmol) in 8 ml of DMF was added isopropylamine (1.89 g, 31.93 mmol) and stirred at room temperature for 4 h. The reaction mixture was poured into10 ml of cold 2 M HCl and stirred for 5 min and 30 ml of cold water was then added and stirred. The precipitate formed was filtered and recrystallized from methanol : hexane mixture, however the filtrate was further extracted with dichloromethane. The organic phase were combined, washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure, which was further recrystallized from chloroform: hexane mixture to give a white needlelike crystals **4** (0.34 g, 30.27%), mp 176-178^{o}C. IR (KBr,ν_{max}, cm^{-1}): 3611 (NH), 1649 (C=O), 1526 (C-N), 770 (1,2-disubstitution).
^{1}H NMR (250MHz, DMSO-d$_6$, δ): 1.13-1.03 (d, 6H, J = 6.6Hz 2 X C**H**$_3$), 1.40-1.38 (d, 6H, J =6.9Hz, 2 X CH$_3$), 4.03-3.95 (sext, 1H, J = 7.6Hz -CH), 4.43-4.32 (quint, 1H, J = 6.9Hz -CH), 7.84-7.81 (m, 2H, Ar-H), 8.08 (d, 1H, J= 7.7Hz, NH).
^{13}C NMR (63MHz, DMSO-d$_6$, δ): 19.8 (2 X CH$_3$), 22.2 (2 X CH$_3$), 40.9 (-CH), 42.2 (-CH), 122.8, 127.6, 129.0, 131.5, 131.7, 134.3, 136.2 (Ar-C), 167.3 (C=O), 165.8 (C=O); MS (m/z, 70eV): 248.1 (5%)[M^{+}], 247.0(16), 190.0(21), 177.1(28), 162.1(10), 148.1 (100), 130.1(72), 105.1(14), 92.1(12), 77.1(10), 58.1(26).
Elemental Analysis: Found (%): C, 67.71; H, 8.06; N, 11.49; $C_{14}H_{20}N_2O_2$. Anal. Calcd. (%): C, 67.52; H, 8.22; N, 11.08.

4-Benzamido-butyric acid –2-(2-methylethyl) carboxamide (5): To a solution of 4-phthalimido butyric acid (1.5 g, 6.43 mmol) in 10 ml of DMF was added isopropylamine (2.6 g, 45.02 mmol) and stirred at room temperature for 30 min. The reaction mixture was poured into 10 ml of cold 2 M HCl and stirred for 5 min and 30 ml of cold water was then added and stirred. This was then extracted with chloroform (3X 20 ml). The organic phase were combined, washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure, which was recrystallized from dichloromethane to give a white needlelike crystals **5** (1.20 g, 95.77%), mp 123-125^{o}C.

IR (KBr,ν_{max}, cm^{-1}): 3743, 3616 (NH), 3418 (OH), 1721 (COOH), 1620 (C=O), 1516 (C-N), 1390 (C=C), 770 (1,2-disubstitution).
^{1}H NMR (250MHz, DMSO-d$_6$, δ): 1.09-1.12 (d, 6H, J = 6.5Hz, 2 X C**H**$_3$), 1.63-1.80 (dd, 2H, J= 7.2Hz, -C**H**$_2$ -), 1.98-2.12 (q, 2H, J= 7.2Hz, HN-C**H**$_2$-), 3.10-3.20 (sext, 1H, J= 6.5Hz, -C**H**), 3.52 -3.57 (t, 2H, J= 7.1Hz, C**H**$_2$COOH), 5.69 (brs, 1H, O**H**), 7.84-7.76 (m, 4H, Ar-H), 8.06-8.09 (d, 1H, J= 7.8Hz, N**H**), 8.40 (t, 1H, J = 7.5Hz, N**H**).
^{13}C NMR (63MHz, DMSO-d$_6$, δ): 20.9 (-2 X CH$_3$), 22.1 (CH$_2$), 33.9 (CH$_2$), 38.4 (CH$_2$), 42.2 (-CH), 122.8, 131.5, 134.2, 136.1(Ar-C), 167.3(C=O), 167.8(C=O), 175.4 (COOH).
MS (m/z, 70eV): 288.3(M^{+}-4)(2%), 282.7(3), 216(12), 214(60), 187.1(64), 174.1(86), 173.1(100), 160.1(98), 147.1(37), 133.1(51), 104.1(30), 77(49), 51.1(29).
Elemental Analysis: Found (%): C, 62.03; H, 6.99; N, 9.72; $C_{15}H_{20}N_2O_4$. Anal. Calcd. (%): C, 61.63; H, 6.50; N, 9.38.

Pharmacological evaluation

Swiss mice (20-25 g) and wistar rats (180- 230 g) of either sex kept at the laboratory Animal home of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water (test compounds were administered orally by gavage in 5% Tween 80 suspensions at different dose level). Ethical approval was obtained from the Animals Use and Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Anti-inflammatory activity

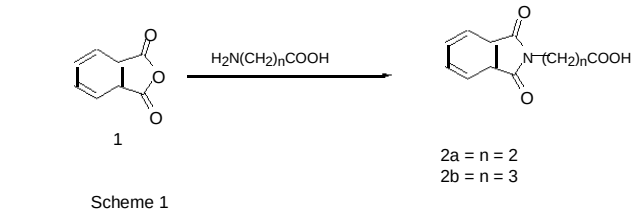
Anti-inflammatory activity was measured using carrageenan-induced rat paw oedema assay (Winter et al., 1962; Adeyemi et al., 2002). Groups of 5 rats of both sexes (pregnant females excluded) were given a dose of a test compound. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 4 h (Bamgbose and Noamesi, 1981). Two groups of drug treated rats and one control group were used each test day; the mean paw oedema value for the test group being compared with its mean value for the control group for that day.

Anti-inflammatory activity (Duffy et al., 2001) was measured as the percentage reduction in oedema level when drug was present, relative to control as shown in Table 2.

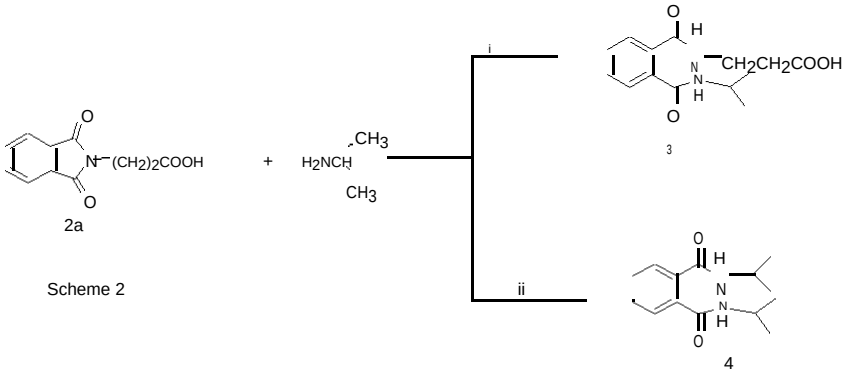
Table 1. Effect of the test compounds on acetic acid-induced writhing test.

Compounds	Dose (mg/kg) p.o.	Number of writhings (per 20 min)	% Inhibition
Control	-	31.17 ± 0.79	-
Indomethacin	10	9.37 ± 0.59**	69.94
Acetylsalicylic acid	100	12.89 ± 1.02**	58.65
Paracetamol	40	17.77 ± 0.82*	42.99
	25	22.61 ± 1.81	27.50
3	50	18.25 ± 2.25**	41.45
	100	16.25 ± 2.18**	47.87
	20	22.53± 1.30	26.76
4	40	18.00 ± 2.86*	42.25
	80	3.60 ±1.36**	88.45
	20	21.50± 1.32*	31.02
5	40	18.25 ± 1.03*	41.45
	80	12.50 ± 0.87**	59.89

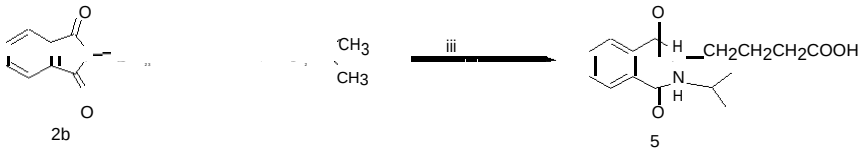
Values are mean ± S.E.M * P< 0.05, **P< 0.001, significantly different from control, paired t- test (n=5), p.o = per oral.



Scheme 1



Scheme 2



scheme 3

i MeOH/CH_2Cl_2 ii DMF, RT iii CH_2Cl_2, RT Or DMF, RT

Figure 1. Synthesis of phthalimido alkyl acids and carboxamides. 3-Phthalimidopropionic acid (2a) and 4-Phthalimidobutyric acid (2b) were treated with isopropylamine at room temperature using different solvents (dimethylformamide, dichloromethane and methanol) as the reaction medium and these further produced 3-benzamido-propionic acid-2- (2-methylethyl)-carboxamide (3), benzamido-N,N-bis-(2-methylethyl)-carboxamide (4) and 4-benzamido-butyric acid-2-(2-methylethyl)-carboxamide (5).

Table 2. Carrageenan rat paw oedema anti-inflammatory activity of the test compounds.

Compounds	Doses mg/kg (p.o.)	Change in paw oedema mean ± SEM (mm)	% oedema inhibition relative to control at the 3rd hour
Control 5 % Tween 80	0.3ml	4.62 ± 2.67	-
Indomethacin	10	1.21 ± 2.36**	73.81
3	25	4.40 ± 2.79	4.76
	50	3.30 ± 1.59*	28.57
	100	3.01 ± 1.03*	34.85
4	20	3.05 ± 2.01*	33.98
	40	2.08 ± 2.24**	54.98
	80	1.87 ± 1.79**	59.52
5	20	3.48 ± 2.01*	24.68
	40	3.01 ± 1.01*	34.85
	80	2.87 ± 2.65*	37.88

Values are mean ± S.E.M; * P< 0.05, ** P< 0.001, significantly different from control, paired t- test (n=5), p.o = per oral.

Activity = 100 – [100 x (average drug treated /average for control)]. Indomethacin (10 mg/kg) was administered orally as reference drug while 5% Tween 80 was used as negative control.

Antinociceptive activity

The methods of Koster (1959) and Adeyemi (2004) were employed. Group of 5 mice of both sexes (pregnant females excluded) were given a dose of a test compound by gavage. After one hour the animals were injected intraperitoneally with 0.2 ml/mouse of 0.6% acetic acid solution (in normal saline) and writhes were counted during the following 20 min. 5% Tween 80 was used as the negative control while acetylsalicylic acid (100 mg/kg p.o), paracetamol (40 mg/kg p.o) and indomethacin (10 mg/kg p.o) were used as reference drugs. The total number of constrictions was summed for five mice in each group analgesic activity were recorded as the percentage inhibition of abdominal constrictions when drug was present compared with control group (Amir and Kumar, 2005)

% Analgesic activity = (n – n'/n) x 100

Where n = mean number of writhes of control group and n' = mean number of writhes of test group.

Statistical analysis

All data were expressed as mean ± SEM and analysed by student's t-test

RESULTS AND DISCUSSION

Chemistry

The synthesis of phthalimido alkyl acids is depicted in scheme 1 as describedin Figure 1 (scheme 1) as described previously (Usifoh et al., 2001). The reaction of the amino acids with phthalic acid anhydride (1) with concomitant removal of water produced phthalimido alkyl acids (2a - b). When 2a was treated with isopropylamine in MeOH : CH_2Cl_2 mixture it yielded compound 3, but in DMF it afforded 2-Benzamido-2- methylethane- 2-(2-methylethyl)-carboxamide (4) which shows that the isopropylamine completely displace the aliphatic carboxylic acid group. This could be attributed to the fact that excess amine was used i.e ratio of 2a: isopropylamine (1:7). A unique product was obtained when 2b and isopropylamine (1:7) react in the presence of dimethylformamide (DMF) to yield compound **5** despite the excess amine used giving 95.77% yield but in the presence of dichloromethane (CH_2Cl_2) it afforded the same product 5 with a low yield (10%). In Figure 1 (scheme 3), the aliphatic carboxylic acid was not replaced by the isopropylamine, which reveal that the number of the methylene group between the –NH- and – COOH affects the stability of the products formed. The reaction time for scheme 3 was 30 min while that of scheme 2 (Figure 1) was 3-4 h. The rate of reaction is affected by the solvent used because of the varying abilities of solvents to solvate reagents and transition state. Solvation refers to specific interactions between solvent molecules and dissolved reagents and or transition state. These interactions are hydrogen bonding, dipole-dipole and ion-dipole interactions.Hence the products formed and the rate of reaction is however affected by the solvent used.

Pharmacology

The *in vivo* anti-inflammatory and analgesic activity of the carboxamides (3, 4 and 5) were determined. Anti-inflammatory activity was measured by means of the carrageenan – induced rat paw oedema. These results are shown in Table 2. 2-Benzamido- 2-methylethane-2-(2-methylethyl)-carboxamide (4) at 80 mg/kg produced the highest activity (60% inhibition) among the compounds

screened, but is less active when compared to the reference drug, indomethacin, which at 10 mg/kg causes 74% inhibition. The effect of the compounds (3, 4 and 5) on carrageenan induced paw oedema was most pronounced at the third hour of inflammatory response, which corresponds to the phase of prostaglandin release (Dannhardt and Kiefer, 2001).

The *in vivo* analgesic activity of compounds 3, 4 and 5 were determined using mouse writhing assay which is a useful test for evaluating mild analgesic NSAIDs and the result obtained are summarized in Table 1. The most active compound is 4, which exhibited 88.45% inhibition at a dose of 80 mg/kg compared to compounds **3** and **5** and the reference drugs (indomethacin, acetylsalicylic acid and paracetamol) used. The percentage inhibition of 4 increases geometrically as the concentration increases, which shows that it has a dose-dependent effect but this was not observed with compounds 3 and 5**.** From the results in Table 1, it could be deduced that not all free acid containing compounds have pronounced analgesic activity when compared to non-free acid containing compounds. Thus the antinociception may not necessarily be due to the acidic moiety present in the compound. From this assay it suggests that the analgesic effect may be peripherally mediated (Santos et al., 1994).

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