ORIGINAL ARTICLE

REACTIONS OF PHTHALIMIDES WITH 1-METHYLETHYLAMINE: ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF RESULTING CARBOXAMIDES

L. O. OKUNROBO, C. O. USIFOH AND S. O. OKPO*

*Departments of Pharmaceutical Chemistry and *Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*

ABSTRACT

The reaction of phthalimide derivatives (**1a-c**) with 1-methylethylamine in dimethylformamide (DMF) at room temperature afforded benzamido-N-prop-2-ynyl-2-(2-methylethyl)-carboxamide (**3a**), benzamido-N-cyclopentyl-2-(2-methylethyl)-car-boxamide (**3b**) and benzamido-N-benzyl-2-(2-methylethyl)-carboxamide (**3c**), respectively. In the carrageenan induced paw oedema test for anti-inflammatory activity, **3a** (50 mg/kg) decreased the inflammatory response by 55% after 3 hrs while 3b and **3c** exhibited significant reduction in the oedema level. The compounds **3a**, **3b** and **3c** exhibited analgesic activities with **3b** producing 76% inhibition. The activities were dose – dependent.

Keywords: Carboxamides, synthesis, analgesic activity, rat paw oedema

Corresponding author: Cyril O. Usifoh, Ph.D, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Phone: +234-8035086361 - e-mail: csusifoh@yahoo.com

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to alleviate mild to moderate pain and in the treatment of inflammatory conditions such as arthritis and spondylitis. However, there are over twenty NSAIDS regularly prescribed in the clinics with undesirable side effects hence there is a continuous search for new and safe drugs (Duffy *et al*., 2001).

A number of new carboxamides have previously been synthesised and screened for various pharmacological properties including anti-inflammatory and analgesic activities. Some novel carboxamides were recently found to have high affinity for dopamine D3 receptors and could be used in the evaluation of animal models of cocaine abuse and consequently assist in elucidating the role of D3 receptors in drug reinforcement *in vivo* (Grundt *et al.*, 2005). Mosapride, a carboxamide and 5-HT4 agonist enhances intrinsic rectorectal and rectoanal reflexes after removal of extrinsic nerves in guinea pigs (Kojima *et al.*, 2005). Carboxamides have also been reported to have weak cholinesterase inhibitory properties but protect cholinesterase *in vitro* from stronger inhibitors like dichlorvos (Petroianu *et al.*, 2005). Iodobenzamides have also been shown to possess anti-fungal activities towards some phytopathogenic fungal strains (Raffa *et al.,* 2002). Furthermore, novel inhibitors representing a diverse range of chemical scaffolds including a series of halogenated phenyl benzamides have been screened as potent and selective inhibitors of plasmodium falciparum dihyroorotate dehydrogenase making the enzyme a strong candidate for the development of new anti-malarial compounds (Baldwin *et al.*, 2005).

The synthesis, anti-inflammatory and analgesic activities of N-heterocyclic carboxamides of thiopyrano-1,2-benzo-thiazine (Schiaffella *et al.*, 1989), N-substituted-(indol-2-yl) and (indo-3-alkyl) carboxamides (Breteche *et al.*, 2002), pyrazole carboxamides (el-Hawash and el-Mallah, 1998) and 3,5-pyrazolidines bearing small-ring cycloalkyl groups (Bellora *et al.,* 1981) have been well documented.

In the present study, carboxamides synthesized from the reaction of phthalimide derivatives with 1-methylethyl-amine, were evaluated for possible anti-inflammatory and analgesic activities.

MATERIALS AND METHODS

Experimental

Melting points were determined with a Kofler melting point apparatus and are uncorrected. IR spectra were recorded on a Buck scientific IR M500 instrument. NMR spectra were recorded on a Varian Gemini 200 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane. Mass spectra were acquired on a Finnigan MAT 44S mass spectrometer operating at 70eV. Elemental analyses obtained for the compounds were within ± 0.4% of the theoretical values. Analytical thin layer chromatography (TLC) on silica gel 60 F_{254} plates from Merck (Darmstadt, Germany) was used to monitor the reactions. The starting materials were obtained from commercial sources and used without purification.

Chemistry

Synthesis of Benzamido-N-prop-2-ynyl-2-(2-methylethyl)-carboxamide (**3a**)

To a stirred solution of N-prop-2-ynylphthalimide (1g, 5.40 mmol) in 8 ml of dimethylformamide, 1-methylethylamine (2.23 g, 37.79 mmol) was added. After stirrng at room temperature for 19 hours, the mixture was poured into ice-cold 2M HCl (2-3 ml) and 40 ml of cold water and extracted with ethylacetate (3x20 ml). The organic phases were combined, washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was crystallized from chloroform/hexane mixture to provide compound (**3a**) as needles (75%, 0.98 g), mp 161–162^oC; IR (KBr, cm $^{-1}$) 3743, 3617 (NH), 3244 (C≡CH), 2180 (C≡C), 1637 (C=0), 1541 (C=C), 776 (1,2- disubstitution); ^{1}H NMR ($CDCl_3$): δ = 1.18-1.21 (d, J = 7.5Hz, 6H, 2XC**H**$_3$), 2.24 – 2.26 (t, J = 5.1Hz, 1H, ≡ C**H**), 4.04-4.09 (t, J = 13.2Hz, 2H, -C**H**$_2$-), 4.12-4.21 (m, 1H, -C**H**), 6.72- 6.75 (d, J =7.5Hz, 1H, **H**N-CH), 7.38 – 7.42 (m, 2H, Ar-H,) 7.48-7.56 (m, 2H, Ar –H), 7.75 (t, J = 5.7Hz ,1H, N**H**-CH$_2$-); ^{13}C NMR ($CDCl_3$): δ = 22.43 (CH_3), 29.65 (CH_2), 42.27 (CH), 71.63(C≡C), 79.19 (C≡C), 128.22, 128.71, 129.90, 129.98, 133.45, 135.65 (Ar –C), 168.43 (C=O), 168.72 (C=O); MS (70eV, m/z); 244.1[M^+] (4.2%), 243.1[M^+ -1] (11.5), 189.1 (34.2), 186.1 (53.1), 148.1 (52.9), 143.1 (24.5), 130.2 (100), 115.1 (32.3), 105.2 (19.6), 77.1 (25.5), 58.1 (25.6); $C_{14}H_{16}N_2O_2$ (244.293) (Found: C 68.71, H 6.52, N 11.28. Calcd: C 68.83, H 6.60, N 11.47).

Synthesis of Benzamido-N-cyclopentyl-2-(2-methylethyl)-carboxamide (**3b**)

To a stirred solution of N-cyclopentylphthalimide (1.0 g, 4.64 mmol) in 10ml of dimethylformamide was added 1-methylethylamine (1.92 g, 32.52 mmol) and stirred at room temperature for 36 hours. The mixture was poured into ice-cold 2M HCl (2-3 ml) and 50 ml of cold water and extracted with ethylacetate (3x20 ml). The organic phases were combined, washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by recrystallising in methanol to obtain white crystals (**3b**) (1.08 g, 85%), mp 203-205^oC; IR (KBr, cm $^{-1}$) 3737, 3610 (NH), 1703 (C=O), 1637 (C=C), 771 (1,2-disubstitution). ^{1}H NMR ($CDCl_3$): δ = 1.22-1.24 (d, J = 6.6Hz, 6H, 2XC**H**$_3$), 1.46-1.53(m, 4H, 2XCH$_2$-cyclopentyl), 1.62-1.74 (m, 2H, -C**H**$_2$-cyclopentyl), 2.00-2.07(m, 2H, -C**H**$_2$-cyclopentyl), 4.13-4.22 (m, 1H, - C**H**-), 4.26-4.40

(sextet, 1H, -C**H**-cyclopentyl), 6.58(t, 1H, N**H**-cyclopentyl), 6.81(d, 1H, -N**H**-CH), 7.42-7.45(m, 2H, Ar-H), 7.53-7.58 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$);: δ = 22.55 (CH_3), 23.80 (CH_2), 33.01, 42.25 (CH), 51.88, 128.23, 128.48, 130.03, 134.56,134.66 (Ar –C), 168.38 (C=O), 168.62 (C=O); MS (70eV, m/z); 274.12 [M^+] (2.6%), 215.09(7.9), 190.04(22.7), 149.04(8.8), 148.03(100), 130.14(80.8), 92.15(11.5), 77.12(11.3), 76.05(6.3), 67.06(7.8), 66.08(3.0); $C_{16}H_{22}N_2O_2$ (274.364) (Found: C 69.86, H 7.90, N 10.04. Calcd: C 70.04, H 8.08, N 10.21).

Synthesis of Benzamido-N-benzyl-2-(2-methylethyl)-carboxamide (**3c**)

To a solution of N-benzylphthalimide (1.0 g, 4.21 mmol) in 8 ml dimethyformamide, was added 1-methylethylamine (1.74 g, 29.50mmol), and stirred at room temperature for 8 hours. The mixture was poured into ice cold 2M HCl (2-3 ml) and 30 ml of cold water, stirred, and extracted with ethyl acetate (3x20ml). The organic extract were combined, washed with brine and dried over $MgSO_4$ (anhydrous) and evaporated under reduced pressure. The residue was purified by crystallization from methanol to obtain white needle like crystals of 3**c** (1.01g, 81**%),** mp 129-130^{o}C; IR (KBr, cm^{-1}): 3737, 3617 (NH), 1625 (C=O), 1469 (CH_2) 771 (1,2-disubstitution); ^{1}H NMR (DMSO d_6): δ = 1.10-1.13 (d, J =7.5Hz, 6H, 2XCH_3), 3.92-4.08 (m, 1H, -C**H**), 4.43-4.46 (d, J=6.0Hz, 2H, -C**H**$_2$) 7.28-7.38 (m, 5H, Ar'-H), 7.46-7.51(m, 4H, Ar-H), 8.25-8.40(d, J = 12.5Hz, 1H, **H**N-CH), 8.81-8.89 (t, J = 8.4Hz 1H, **H**N-CH_2-); ^{13}C NMR (DMSO d_6): δ = 22.14 (CH_3), 42.41 (CH_2), 54.87 (CH), 126.63, 127.57, 127.67, 128.15 (Ar' –C), 129.08, 129.30, 135.86, 136.67, 139.40(Ar-H) 167.29 (C=O), 168.14 (C=O); MS (70eV, m/z); 296.97[M^+] (5.5%), 237.1 (31.3), 208.2 (21.7), 190.06 (100), 180.1 (27.1), 148.1 (83.0), 130.1 (43.7), 106.1 (16.8), 104.2 (25.4), 91.1 (98.7), 77.1 (17.8); $C_{18}H_{20}N_2O_2$ (296.369) (Found:C 72.75, H 6.56, N 9.39. Calcd: C 72.95, H 6.80, N 9.45).

Pharmacological evaluation

Swiss mice (25-30 g) and wistar rats (200-250 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 15% tween 80 suspension at different dose levels).

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 one-hour 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 four hours (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 1. Activity = 100 – (100 x (average drug treated/average for control).

Indomethacin (10 mg/kg) was administered orally as reference drug while 15% tween 80 was used as negative control.

Analgesic activity

The method of (Koster *et al.,* 1959) and (Adeyemi *et al.,* 2004) were employed. Groups 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 %v/v acetic acid solution (in normal/saline) and writhes were counted during the following 20minutes. 15% tween 80 was used as the negative control while acetylsalicylic acid (100 mg/kg and 50 mg/kg p.o) was used as reference drug. The total number of constrictions was summed for five mice in each group. Analgesic activities were recorded as the percentage inhibition of abdominal constrictions when drug was present compared with control group (Duffy *et al.,* 2001).

% Inhibition = 100- (100 x average drug response/average control response). All data were expressed as mean ± SEM and analysed by student's t-test

DISCUSSION AND RESULTS

Chemistry

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 easily encounted. The attack on the carbonyl carbon by the nucleophilic reagents such as amines is possible resulting in C-N bond cleavage to provide the corresponding diamides. Thus the phthalimide derivatives (**1a-c**) with different substituents at the nitrogen atom were transformed to the corresponding carboxamides (**3a-c**) as shown in scheme 1 with 1-methylethylamine. Generally the percentage yields of 3a-c were high but the reaction time and melting point were in the following order; (**3c < 3a < 3b**). This sequence may be due to the electron density of the substituent and it may be postulated that the higher the electron rich substituent the faster the reaction.

The 1R data clearly reveals the presence of -NH between 3743-3610 cm^{-1} for compounds **3a-c**. The proton NMR shows that NH attached to the amine appears at the downfield region as a doublet. The two methyl groups attached to the –CH also appears as a doublet. The above spectroscopic data differentiate these carboxamides (**3a-c**) from the starting materials.

Pharmacology

The *in vivo* anti-inflammatory and analgesic activity, of the carboxamides (**3a-c**) were determined. Anti-inflammatory activity was measured by means of the carrageenan – induced rat paw oedema. These results are shown in table 1. The compound **3a** at 50 mg/kg producing 55% inhibition was the most active but less active compared to the reference drug indomethacin which at 10 mg/kg causes 74% inhibition.

Table 1: Carrageenan rat paws oedema: anti-inflammatory activity of the test compounds

Compounds	Doses mg/ kg P.O	% Inhibition in oedema level at the 3rd hr.
3a	50	54.98
	25	-13.42
3b	40	24.68
	20	-21.43
3c	40	37.45
	20	-1.08
Indomethacin	10	73.81

The minus sign of the percent inhibition in some cases denotes that the size of the oedema exceeded the size of the respective control series.

The effect of the compound (**3a-c**) 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 results obtained for compound **3a-c** show that these possess anti-inflammatory activity, which is probably consistent with its ability to inhibit prostaglandin synthesis in platelet (Naik *et al.,* 1972).

Analgesic activity was determined using mouse writhing assay, which is a test useful for evaluating mild analgesic NSAIDS, and results obtained are summarized in table 2.

The most active compound was **3b** with 76% inhibition at dose of 40 mg/kg followed by **3c** and **3a**. From this assay, it be suggested that the analgesic effect of the compounds (**3a-c**) may be peripherally mediated (Santos *et al.,* 1994).

The results obtained from both assays show that the compounds possess a dose-dependent anti-inflammatory and analgesic effects.

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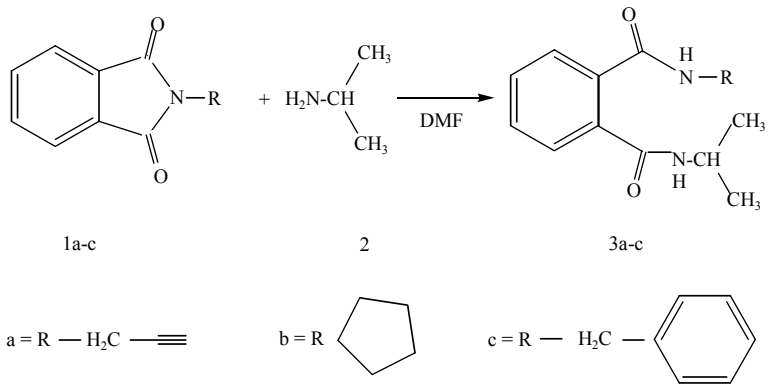
Table 2: Acetic acid writhing test in mice for the test compounds

Compounds	Doses mg/kg P.O	No of writhes (per 20 mins)	% Inhibition
Control	-	63.11± 4.46	-
Acetylsalicylic Acid (ASA)	100	30.80 ±1.83[a]	52.00
	50	47.60 ± 1.54[b]	25.23
3a	100	36.80±3.09[a]	41.69
	50	54.40 ±1.72[b]	13.80
	30	62.60 ±1.54[b]	1.12
3b	40	15.00± 0.71[a]	76.23
	20	19.60 ±1.50[a]	68.90
	10	45.20 ±2.22[b]	28.38
3c	80	12.20 ±1.77[a]	80.66
	50	28.00 ±1.92[a]	55.63
	25	43.80±3.76[b]	30.59

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



Scheme 1

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