Ring Opening of Phthalimide Derivatives with Benzylamine: Formation of Carboxamides and their Pharmacological Evaluation

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The ring opening of phthalimide derivatives viz N-cyclopentylphthalimide (1a), N-benzylphthalimide (1b), N-prop-2-ynylphthalimide (1c), 1-phthloylamino-3-[4-(2-methoxyphenyl)-piperizin-1-yl]-propane(1d) and 1-phthloylamino-4-[4-(2-methoxyphenyl)-piperizin-1-yl]-butane (1e) was accomplished using benzylamine in dimethylformamide (DMF) at room temperature to afford the corresponding carboxamides: benzamido-cyclopentane-2-(N-benzyl)-carboxamide (3a) benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide (3b) and 3-benzamido-prop-2-yne-2-(N-benzyl)- carboxamide (3c) and were unequivocally characterized by infrared, nuclear magnetic resonance, mass spectrometer and elemental analyses. The products obtained were screened for antiinflammatory and analgesic properties using carrageenan-induced rat paw oedema assay and acetic acid-induced writhing test, respectively. The most active compound was 3b for the antiinflammatory activity assay and for the analgesic activity test the most active compound was 3a. The activities were dose-dependent. All the compounds tested showed better analgesic activity than acetylsalicylic acid.

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Non-steroidal antiinflammatory 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 the search for newer drugs[1]. A good number of new carboxamides and their derivatives have been synthesized and screened for various pharmacological properties including antiinflammatory and analgesic activities. New antiinflammatory agents 4-hydroxy-2-(1H)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides were synthesized and screened for antiinflammatory activity[2]. Other researchers have also synthesized and evaluated carboxamide derivatives for their antiinflammatory and analgesic activities; for example N-heterocyclic carboxamides of thiopyrano-1,2-benzothiazine[3], N-substituted-(indol-2-yl) and (indo-3-alkyl) carboxamides[4], and pyrazole carboxamides[5]. Carboxamides have also been reported to have weak cholinesterase inhibitory properties but protect cholinesterase *in vitro* from stronger inhibitors like dichlorvos[6].

In the present study, new carboxamide derivatives were synthesized via the ring opening of phthalimide derivatives with benzylamine and screened for antiinflammatory and analgesic activities.

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 analyses were performed at the analytical Laboratory, University of Münster, Germany and agreed favourably with the theoretical values. Analytical thin layer chromatography (TLC) was used to monitor the reactions.

General synthetic procedure:

To a stirred solution of phthalimide derivatives in 10 ml of dimethylformamide or dichloromethane was added benzylamine drop wise and stirred for 7-21 h at room temperature. The reaction mixture was poured into 5 ml of 2 M HCl and 30 ml of cold water and stirred for 5 min and then extracted with dichloromethane or ethyl acetate. The organic phase was combined, washed with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was

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purified by recrystallization from appropriate solvent or by column chromatography to afford the desired products.

Benzamido-cyclopentane-2-(N-benzyl)-carboxamide (3a):

To a stirred solution of N-cyclopentyl phthalimide 1a (0.30 g, 0.00139 mol) in 10 ml of dimethylformamide was added benzylamine (0.45 g, 0.00418 mol) and stirred for 7.5 h and treated as in the general procedure to give a crude product which was purified by column chromatography (CH_2Cl_2:MeOH 9:1) and recrystallized from methanol to afford white needles of benzamido-cyclopentane-2-(N-benzyl)-carboxamide; (0.27 g, 60%), mp**:** 100-102^0. IR (KBr): 3284 (NH), 3077, 2941(C-H), 1703 (C=O), 1636 (C=C), 1545 (C-N), 770 (1,2-disubstitution) cm^{-1}; ^{1}H NMR (250MHz, DMSO-d_6) δ: 1.48-1.63 (m, 2H, -CH_2-cyclopentyl), 1.79-1.99 (m, 2H, -CH_2-cyclopentyl), 4.08-4.15 (m, 1H, -CH-cyclopentyl), 4.41-4.44 (d, 2H, J=5.99Hz, -CH_2-Ar), 4.48-4.58 (t, 4H, J=8.23Hz, 2x -CH_2-cyclopentyl), 7.44-7.49 (dd, 2H, J=10.25Hz, Ar'-H), 7.19-7.35 (m, 3H, Ar'H), 7.84 (dd, 4H, J=7.50Hz, Ar-H), 8.19 (d, 1H, J=7.50Hz, NH) 8.75 (t, 1H, J=5.00Hz, NH); ^{13}C NMR (63MHz, DMSO-d_6) δ: 23.5 (CH_2), 24.7 (CH_2), 29.2(CH_2), 32.0 (CH_2), 42.4 (CH_2-Ar'), 50.7 (-CH-), 122.8, 126.6, 127.1, 127.5, 127.8, 128.1 (Ar'-C), 129.0, 129.3, 131.4, 135.7, 136.6, 139.3 (Ar-C), 167.8 (C=O), 168.1 (C=O); MS m/z: (70eV): 322.0 (4.5%, M^+), 237.9 (M^+-84,11.2), 216.0 (M^+-106, 100), 208.1(15.6), 180.1(17.9), 148.0 (53.9), 91.0 (81.6), 65.1 (14.8); Elemental Analysis**:** Calcd. for $C_{20}H_{22}N_2O_2$ (322.407): C, 74.51, H, 6.88, N, 8.69, Found: C, 74.60, H, 6.99, N, 8.80

Benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide (3b):

To a solution of N-benzylphthalimide 1b (1.00 g, 0.00421 mol) in 10 ml of dimethylformamide was added benzylamine (1.36 g, 0.01264 mol) stirred for 18 h and treated as in the general procedure to give a crude product and purified by recrystallization from methanol to afford white needle like crystals of benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide**:** (0.87 g, 60%), mp 168-170°. IR (KBr): 3247 (NH), 3077 (C-H), 1636 (C=O), 1560 (C=C), 797 (1,2-disubstitution) cm^{-1}; ^{1}H NMR (250MHz, DMSO-d_6) δ: 4.41-4.44 (d, 2H, J=5.97Hz, -CH_2 –Ar'), 7.24-7.40 (m, 5H, Ar'-H), 7.50-7.52 (dd, 4H, J=2.01Hz, Ar-H), 8.84 (t, 1H, J=5.96Hz, -NH), ^{13}C NMR (63MHz, DMSO-d_6) δ: 42.4 (-CH_2-Ar') 126.6, 127.6, 128.2, 128.6, 129.3 (Ar'-C), 136.2, 138.9, 139.4 (Ar-C), 168.2 (C=O); MS (m/z, 70 eV): 345.12(M^+ + 1, 2%), 327.0 (2), 238.0 (37), 237.1 (28), 209.1 (11), 180.1 (20), 130.0 (7), 108.0 (16), 106.0 (12), 91.0 (100), 77 (10), 65.0 (12). Elemental analysis: Calcd. $C_{22}H_{20}N_2O_2$ (344.413): C, 76.72, H, 5.85, N, 8.13, Found: C, 76.55, H, 5.94, N, 8.22

3-Benzamido-prop-2-yne-2-(N-benzyl)-carboxamide (3c):

To a solution of N-prop-2-ynylphthalimide 1c (0.40 g, 0.00216 mol) in 10 ml of dimethylformamide was added benzylamine (0.23 g, 0.00216 mol) and stirred for 21 h and treated as in the general procedure to give a crude product and purified by recrystallization from methanol to afford white needlelike crystals of 3-benzamido-prop-2-yne-2-(N-benzyl)- carboxamide; (0.57 g, 91%), mp: 143-145°. IR (KBr): 3693, 3616 (NH), 3249 (C≡CH), 2160 (C≡C), 1670 (C=0), 1590 (C=C), 766 (1,2- disubstitution) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.12-3.14 (t, 1H, J=2.5Hz, ≡C-H), 3.96-3.99 (t, 2H, J=2.5Hz -CH_2), 4.41-4.43 (d, 2H, J=5.9Hz, -CH_2-Ar), 7.23 - 7.32 (m, 5H, Ar'-H), 7.46 - 7.50 (m, 4H, Ar-H), 7.87-7.89 (t, 1H, J=2.7Hz, NH), 8.70-8.77 (t, 1H, J=5.9Hz, NH); ^{13}C NMR (DMSO-d_6) δ: 28.4 (≡CH), 42.4 (-C≡), 73.0(CH_2-), 80.9 (CH_2-), 123.3, 126.6, 127.1, 127.6, 128.2 (Ar'-C), 129.3, 129.5, 134.7, 135.5,136.3,139.9(Ar-C), 167.7 (C=O), 168.0 (C=O); MS: m/z 292.9(21%, M^+), 237.9(5), 180.1(2). Elemental Analysis**:** Calcd. for $C_{18}H_{16}N_2O_2$ (292.337): C, 73.96; H, 5.52; N, 9.58. Found: C, 73.81; H, 5.61; N 9.76.

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 10% Tween 80 suspension 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.

Antiinflammatory activity:

Antiinflammatory activity was measured using carrageenan-induced rat paw oedema assay[7,8]. Group 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 4 h[9]. Two groups of drug treated rats and one control group was used each test day and the mean paw oedema value for the test group being compared with its mean value for the control group for that day.

Antiinflammatory activity[1] was measured as the percentage

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reduction in oedema level when drug was present, relative to control as shown in Table 1. Indomethacin (10 mg/kg) was administered orally as reference drug while 10% Tween 80 was used as negative control. All data were expressed as mean±SEM; the student's *t*-test was applied to determine the significance of the difference between the control group and treated group.

Analgesic activity:

Acetic acid induced writhing test[10,11] was performed by an intraperitoneal injection of 0.6% acetic acid solution in a volume of 0.2 ml/mouse. In each group five Swiss mice of both sexes (pregnant females excluded) were kept and given a dose of a test compound by gavage. Screening of analgesic activity was performed after p.o. administration of the test compounds at different dose level. After 1 h of drug administration 0.2 ml of 0.6% acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 20 min after acetic acid injection. Indomethacin (10 mg/kg) and acetylsalicylic acid (100 mg/kg) were administered orally as reference drug while 10% Tween 80 was used as negative control. The analgesic activity[12] was expressed in term of % inhibition. % Analgesic activity=(n-n'/n)×100, where n=mean number of writhes of control group, n'=mean number of writhes of test group. All data were expressed as mean±SEM; the student's *t*-test was applied to determine the significance of the difference between the control group and mice treated with the test compounds.

TABLE 1: ANTIINFLAMMATORY ACTIVITY OF THE TEST COMPOUNDS IN CARRAGEENAN RAT PAW OEDEMA MODEL

Compounds	Doses mg/kg (p.o)	Change in paw oedema mean±SEM (mm)	% Oedema inhibition relative to control at 4 h
Control 10% Tween 80	0.3 ml	4.51±1.35	-
Indomethacin	10	1.42±0.12*	68.51
3a	20	3.75±0.48	16.85
	40	2.13±0.32*	52.88
	80	1.50±0.25*	66.74
3b	20	2.50±0.35*	44.56
	40	2.17±2.45*	51.88
	80	1.67±0.58*	62.97
3c	20	4.00±0.41	11.31
	40	2.50±1.92*	44.57
	80	2.13±1.79*	52.88

Values are mean±SEM, *significantly different from control at p<0.05, Paired t-test (n=5), p.o.= per oral, 3a= benzamido-cyclopentane-2-(N-benzyl)-carboxamide, 3b= benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide and 3c= 3-benzamido-prop-2-yne-2-(N-benzyl)- carboxamide

TABLE 2: EFFECT OF THE TEST COMPOUNDS ON ACETIC ACID-INDUCED WRITHING TEST

Compounds	Doses in mg/kg (p.o.)	Numbers of writhing (per 20 min)	% Inhibition
Control 10% Tween 80	0.2 ml	56.86±4.21	-
Acetylsalicylic acid	100	22.50±3.07*	60.43
Indomethacin	10	14.80±4.95*	73.97
3a	20	32.75±2.14*	42.40
	40	15.50±4.56*	72.74
3b	20	38.25±3.84	32.73
	40	25.25±2.29*	55.59
	80	19.50±2.90*	65.71
3c	10	44.33±6.18	20.04
	20	42.50±5.69	25.26
	40	21.67±4.06*	61.89

Values are mean±SEM. *significantly different from control at P<0.05, Paired t-test (n=5), p.o. = per oral, 3a= benzamido-cyclopentane-2-(N-benzyl)-carboxamide, 3b=benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide and 3c=3-benzamido-prop-2-yne-2-(N-benzyl)- carboxamide

RESULTS AND DISCUSSION

Phthalimide derivatives (1a-e) were treated with benzylamine in dimethylformamide (DMF) as shown in scheme 1. The reaction of N-cyclopentyl phthalimide (1a) and N-benzylphthalimide (1b) with equimolar or excess benzylamine gave benzamido-cyclopentane-2-(N-benzyl)-carboxamide (3a) and benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide (3b), respectively; but N-prop-2-ynylphthalimide (1c) with equimolar benzylamine gave 3-benzamido-prop-2-yne-2-(N-benzyl)- carboxamide (3c); with excess of benzylamine it afforded (4); however when 1-phthloylamino-3-[4-(2-methoxyphenyl)-piperizin-1-yl]-propane(1d) and 1-phthloylamino-4-[4-(2-methoxyphenyl)-piperizin-1-yl]-butane (1e) were treated with either equimolar or excess of benzylamine to give the anticipated benzamido-N-benzyl-3-propyl-1-[4-(2-methoxyphenyl)-piperizin-1-yl-carboxamide (3d) and benzamido-N-benzyl-4-butyl-1-[4-(2-methoxyphenyl)-piperizin-1-yl-carboxamide (3e) but it afforded a dimmer benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide (4) which was not expected, this shows that benzylamine completely replaced the substituent. The NMR, MS and elemental analysis revealed that (4) was same as (3b). Benzamido-cyclopentane-2-(N-benzyl)-carboxamide (3a), benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide (3b) and 3-benzamido-prop-2-yne-2-(N-benzyl)-carboxamide (3c) were opened products of the starting materials.

Infra-red (1R) data clearly reveal the presence of -NH between 3693-3240 cm^{-1} that was completely absent in the corresponding phthalimide. The proton NMR clearly shows -NH at the down field region. In compound 3a -

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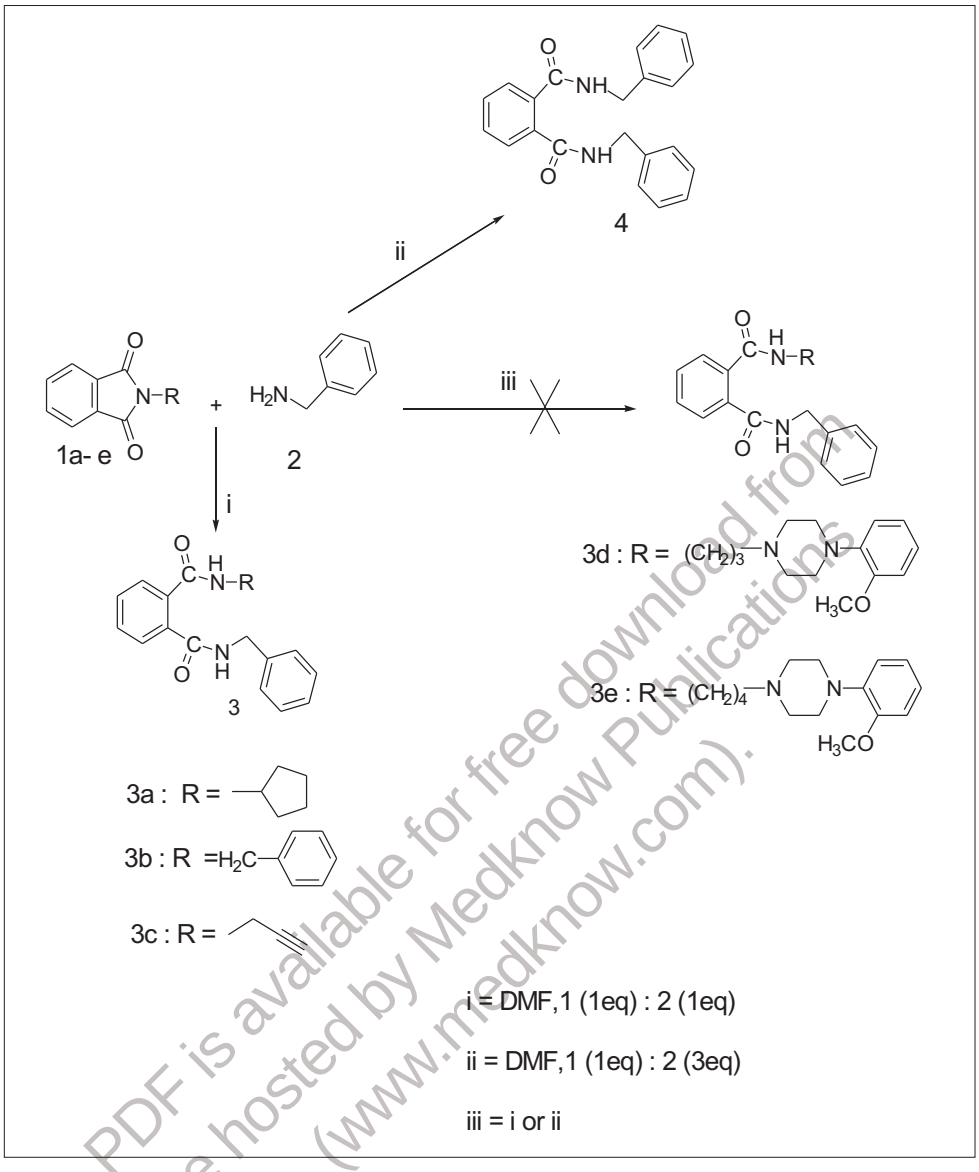

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Scheme 1: Synthesis of carboxamide derivatives 3a, 3b and 3c. 3a= Benzamido-cyclopentane-2-(N-benzyl)-carboxamide, 3b= 4= benzamido-1-phenylmethylene-2-(N-benzyl)-carboxamide and 3c= 3-benzamido-prop-2-yne-2-(N-benzyl)-carboxamide

NH appear as a triplet at about 8.75 ppm and another at 8.19 ppm as a doublet, for 3c -NH appears at between 7.87-7.89 as a triplet and 8.70-8.77 ppm as triplet but for compound 3b -NH appears between 8.82-8.77 ppm as a triplet only, which confirmed di-substitution with benzylamine.

The antiinflammatory activity of the synthesized compounds 3a, 3b and 3c were evaluated by carrageenan-induced rat paw oedema method of Winter *et al.*[7] and Bamgbose and Noamesi[9] the results are shown in Table 1. The compounds were tested at three different dose levels to know if they were dose-dependent; all the test compounds showed activity. At the lowest dose of 20 mg/kg, 3b was most active producing 45% inhibition ($p< 0.05$) while at the highest dose used 80 mg/kg, 3b that was most active produced 67% inhibition ($p<0.05$). It was comparable to the standard drug used (indomethacin 69% inhibition). From the study it shows that the compounds with cyclopentyl and benzyl substituent showed more activity compared to the one with prop-2-ynyl moiety.

Analgesic activity was determined using mouse writhing assay, which is a test useful for evaluating mild analgesic NSAIDs, and the results obtained are summarized in Table 2. At 40 mg/kg, the most active compound is 3a with73% inhibition ($p<0.05$) comparable to indomethacin with 74% inhibition ($p<0.05$), followed by 3c with 62%

activity then 3b with 56% activity. The compounds 3a-c were more potent than acetylsalicylic acid. From this assay it suggests that the analgesic effect of compounds 3a-c may be peripherally mediated[13].

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