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RING OPENING OF 1-PHTHALOYLIMIDO-[4-(2-METHOXYPHENYL) PIPERAZIN-1-YL]ALKYL DERIVATIVES: SYNTHESIS, ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF PRODUCTS

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Abstract: Phthaloylimidoalkyl derivatives (**1a, 1b**) were treated with isopropylamine to give 3-benzamido-1-(4-(2-methoxyphenyl)piperazin-1-yl)]propyl-2-isopropyl carboxamide **(3a)** and 4-benzamido-1-(4-(2-methoxyphenyl)piperazin-1-yl)butyl-2-isopropyl carboxamide (**3b**). The compounds were unequivocally characterized by IR, NMR and MS spectra and elemental analysis. Carrageenan-induced rat paw assay was used to screen **3a** and **3b** for anti-inflammatory activity. 3**b** significantly ($p < 0.001$) inhibited the inflammation caused by carrageenan after 3 h compared to **3a** and indomethacin. Mouse writhing assay was performed to evaluate **3a** and **3b.** It revealed that **3b** (10 mg/kg) produced 71% inhibition when compared to **3a** (40 mg/kg) and acetylsalicylic acid (100 mg/kg). The results show that the length of the alkyl chain that separates the terminal nitrogen atom from the phthalic acid moiety also affects the activity of **3a** and **3b** in a dose dependent manner.

Keywords: Aryl piperizines, ring opening, anti-inflammatory activity, mouse writhing test, isopropylamine.

Compounds containing arylpiperazine moieties constitute a class of important agents with a variety of pharmacological activities acting via neurotransmitter blocker such as 5-hydroxytryptamine antagonist (1) . It has been found that phthalimides and their derivatives have been used as inhibitors of tumor necrosis factor-alpha production (2,3).

In phthalimides, the delocalization of electrons on the two oxygen and nitrogen atoms makes the nitrogen atom nucleophilic and hence it is capable of undergoing nucleophilic substitution reaction. Phthalimides and their derivatives may have their ring opened using amine to obtain the respective carboxamides and many carboxamides have been shown to have analgesic and anti-inflammatory activities. A series of N-pyridinyl-(methyl) quinoline-3-carboxamides have been synthesized and evaluated for its topical anti-inflammatory activity (4). Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation, especially arthritis (5). The aim of this study was to open the ring of some phthalimide derivatives using isopropylamine, screen the carboxamide derivatives formed for anti-inflammatory and analgesic properties and to study the effect of the alkyl chain length that separates the terminal nitrogen atom from the phthalic acid moiety.

EXPERIMENTAL

Chemistry

N-bromoalkylphthalimides used were from Acros Organics, New Jersey, USA, acetylsalicylic acid was from BDH Chemicals Ltd., England and other reagents were from Sigma-Aldrich, Germany.

Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were recorded on a Buck Scientific IR M500 instrument. The NMR spectra were recorded on a Varian Gemini 200 spectrophotometer. Chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were acquired on a Varian MAT 44S mass spectrometer operating at 70eV. Elemental analyses were in agreement with the calculated values. Analytical thin layer chromatography (TLC, silica gel 60 F_{254} plates, Merck, Darmstadt, Germany, mobile phase: ethyl acetate:methanol, 3:1 v/v, visualization: UV 254 nm) was used to monitor the reactions.

Synthesis of phthaloylimidoalkyl derivatives

N-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl-phthalimide **(1a)**

To a refluxing mixture of 1-(2-methoxyphenyl)piperazine (8.3 g, 43.2 mmol) and anhydrous potassium carbonate (12 g, 86.8 mmol) in acetonitrile N-(3-bromopropyl)phthalimide (9.8 g, 36.0

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mmol) was added. The mixture was refluxed for 24 h, filtered hot and the solvent was removed *in vacuo*. Column chromatography (on silica gel 60 F_{254} 0.2-0.05 mm, Merck, Darmstadt, Germany) with dichloromethane as a mobile phase was used to purify the compound, giving yellow oil, which slowly solidified. Recrystallization from ethanol:diethyl ether (1:1, v/v) afforded 13.2 g of (**1a)** (95%), m.p. 100-102^{o}C (lit. (6) 101-102^{o}C).

N-4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl-phthalimide **(1b)**

N-(4-bromobutyl)phthalimide (10 g, 35.5 mmol) and 1-(2-methoxyphenyl) piperazine (8.19 g, 42.6 mmol) were condensed in the presence of anhydrous potassium carbonate (11.78 g, 85.2 mmol) in 150 mL of acetonitrile for 48 h.The yellow oil obtained solidified over 7 days to yield (**1b**) 12.6 g, 90%, m.p. 68-70^{o}C (lit. (6) 69-70^{o}C).

Synthesis of amides

3-Benzamido-1-(4-(2-methoxyphenyl)piperazin-1-yl)]propyl-2-isopropyl carboxamide **(3a)** To a stirred solution of **1a** (1.5 g, 2.64 mmol) in 15 mL of dimethylformamide isopropylamine (2.36 mL, 18.44 mmol) was added at room temperature. The mixture was stirred for 32 h and then poured into 50 mL of cold water and stirred for 10 min. The resulting precipitate was filtered, washed with water, dried and recrystallized from dichloromethane: hexane mixture (1:2, v/v) to give white crystals of (**3a**) (0.46 g, 25%), m.p. 152-153^{o}C.

IR (KBr, cm-^{1}): 3737, 3610 (NH), 1655 (C=O), 1107 (C-O), 758.6 (1,2-disubstitution).

^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.22 (d, *J* = 6.6 Hz, 6H, 2 × CH_3), 1.71-1.78 (quint, *J* = 6.2 Hz, 2H, -CH_2-), 2.46 – 2.56 (t, *J* = 13.7 Hz, 2H, -CH_2-N), 2.59 (br s, 4H, piperizine (2 × CH_2-), 2.83 (brs, 4H, piperizine 2-CH_2-), 3.45-3.52 (q, *J* = 5.9 Hz, 2H, -NH-CH_2-), 3.82 (s, 3H, -OCH_3), 4.14 – 4.22 (m, 1H, -CH), 6.73 – 6.82 (m, 2H, Ar'-H), 6.83 – 6.85 (m, 2H, Ar'-H), 7.37 – 7.43 (m, 4H, Ar-H), 7.64 (d, 1H, *J* = 9.5 Hz, -HN-CH), 8.33 (t, 1H, *J* = 10.1 Hz, NH-CH_2-). ^{13}C NMR ($CDCl_3$) δ (ppm): 22.6, 24.6, 40.4, 42.1, 50.7, 53.3, 55.3, 57.4, 118.2, 120.9, 123.2, 127.5, 128.9, 129.9, 133.9, 134.8, 135.4, 141.0, 152.2 (Ar-C), 167.7 (C=O), 169.3 (C=O); MS m/z: 438 (10%, M^+), 424 (12), 423 (43), 379 (11), 290 (41), 205 (35), 190 (82) 162 (23), 148 (100), 120 (63), 77 (33). Analysis: $C_{25}H_{34}N_4O_3$ (438.572), calc.: C 68.47, H 7.81, N 12.77%; found: C 68.54, H 7.72, N 12.59%;

4-Benzamido-1-(4-(2-methoxyphenyl)piperizin-1-yl)butyl-2-(2-methylethyl) carboxamide **(3b).** To a solution of **1b** (1.00 g, 2.54 mmol) in 10 mL of dimethylformamide isopropylamine (1.53 mL, 17.79 mmol) was added and the reaction mixture was stirred at room temperature for 56 h. The mixture was poured into 50 mL of cold water and stirred for 10 minutes. The precipitate was filtered, washed with cold water and dried. The residue was collected and recrystallized from chloroform: hexane mixture. The filtrate was extracted with ethyl acetate. The various phases were combined, and washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was further recrystallized from chloroform : hexane mixture (1:2, v/v) to give yellowish crystals of **3b** (0.79 g, 69%), m.p. 120-122^{o}C.

IR (KBr, cm-^{1}): 3737, 3616 (NH), 1643 (C=O) 1132 (C-O), 764.7 (1,2 disubstitution).

^{1}H NMR (250MHz, $CDCl_3$) δ (ppm): 1.21-1.23 (d, *J* = 6.6 Hz, 6H, 2 × -CH_3) 1.61 – 1.67 (m, 4H, CH_2-CH_2-), 2.40-2.45 (t, *J* = 13.0 Hz, 2H, -CH_2-N), 2.58 (brs, 4H, piperazine, 2 × CH_2-), 2.84 (brs, 4H, piperazine 2 × CH_2-), 3.37-3.44 (q, *J* = 6.1 Hz, 2H, NH-CH_2-), 3.83 (s, 3H, OCH_3), 4.12-4.26 (m, *J* = 6.6 Hz, 1H, -CH), 6.79-6.85 (m, 2H, Ar'-H) 6.90-6.95 (m, 2H, Ar'-H), 7.38-7.48 (m, 4H, Ar-H) 7.48 (d, 1H, *J* = 5.0 Hz, -HN-CH), 8.10 (t, 1H, *J* = 6.1 Hz, -HN-CH_2).

^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 22.6, 24.5, 27.4, 40.1 42.1, 50.6, 53.3, 55.3, 57.9, 111.1, 118.3, 120.9, 122.9, 127.9, 128.6, 129.8, 130.1, 134.5, 135.2, 141.1, 152.2 (Ar-C), 167.9 (C=O), 169.6 (C=O). MS: m/z 452 (3%, M^+), 438 (M^+-14) (5), 437 (24), 290 (49), 205 (59), 190 (83), 177(70), 160(32), 148 (100), 130 (63), 105 (22) 77 (39). Analysis: $C_{26}H_{36}N_4O_3$ (452.599) calc. C 68.99, H 8.02, N 12.38%; found: C 69.08, H 7.90, N 12.17%.

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).

Carrageenan-induced rat paw edema

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay (7,8). Group of 5 rats of both sexes (pregnant females excluded) were given a dose of a test compound. After 1 h 0.1 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected into the

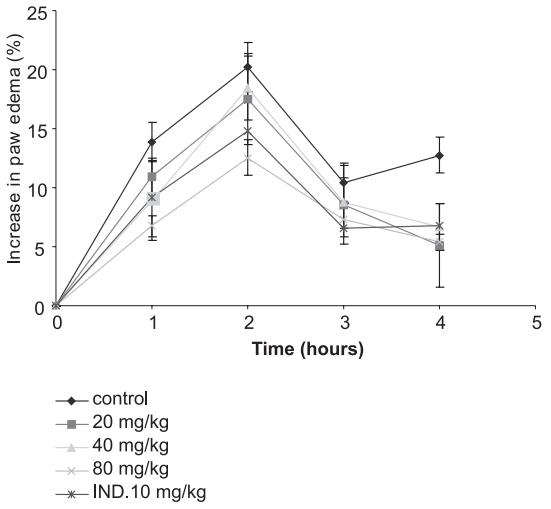


Figure 1. Effect of compound **3a** on carrageenan-induced edema. Each point represents mean ± S.E.M. (n = 5), $p < 0.05$ for the dose of 20 mg/kg, $p < 0.001$ for the doses of 40 and 80 mg/kg, IND. = Indomethacin.

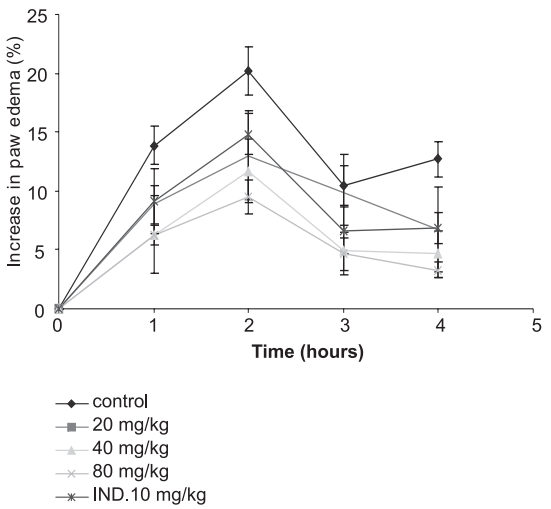
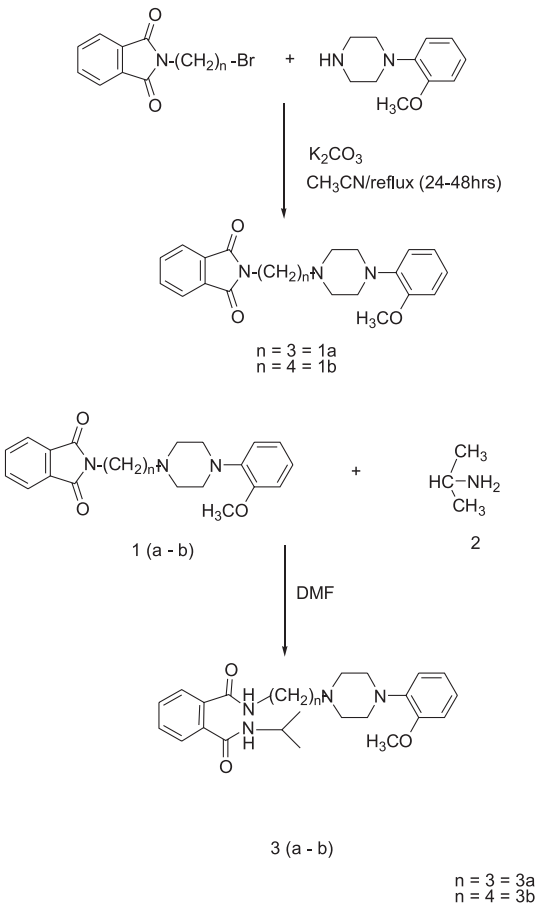


Figure 2. Effect of compound **3b** on carrageenan-induced edema. Each point represents mean ± S.E.M. (n = 5), $p < 0.05$ for the dose of 20 mg/kg, $p < 0.001$ for the doses of 40 and 80 mg/kg, IND.= Indomethacin.

subplantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 4 h (9). Three groups of drug treated rats and one control group were used each test day, the mean paw edema value for the test group being compared with its mean value for the control group for that day. Indomethacin (10 mg/kg) was administered orally as reference drug whereas 15% Tween 80 was used as negative control. The experimental results are shown in Figures 1 and 2 .



Scheme 1.

Mouse writhing assay

The methods of Koster et al. (10) and Adeyemi et al. (11) were employed. Groups of 5 mice of both sexes (pregnant females excluded) were given a dose of a test compound by gavage. After 1 h the animals were injected intraperitoneally with 0.2 mL/mouse of 0.6% acetic acid solution (in 1M saline) and writhes were counted during the following 20 min. 15% Tween 80 was used as the negative control, whereas acetylsalicylic acid (100 g/kg p.o.) was used as a reference drug. The total number of constrictions was summed for five mice in each group and analgesic activities were recorded as the percentage inhibition of abdominal constrictions when drug was present compared with the control group (12):

% inhibition = 100 – [100 × (average drug response/average control response)]

RESULTS AND DISCUSSION

The research work of Glennon et al. (13) revealed that the length of the alkyl chain that sepa-

Table 1. Acetic acid writhing test in mice for the investigated compounds.

Compounds	Doses (mg/kg) p.o.	Number of writhings (per 20 min)	% Inhibition
Control 15% Tween 80	0.2 mL	37.86 ± 5.35	–
Acetylsalicylic acid	100	15.40 ± 1.89**	59
3a	10	15.81 ± 2.56*	58
	25	15.20 ± 3.35**	60
	50	13.60 ± 2.58**	64
3b	10	11.00 ± 1.98**	71
	20	6.40 ± 1.81**	83
	40	4.21 ± 1.16**	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

rates the terminal amine from the phthalimido group is of major importance and a four-carbon chain appears optimal. Alteration of the length of this chain can have a significant influence on affinity for receptor site; decreasing the chain length from four to three carbon atoms can reduce affinity by an order of magnitude and further shortening can have an even more pronounced effect.

Chemistry

The phthaloylimido alkyl derivatives (**1**) were synthesized according to the literature procedure (6) as shown in Scheme 1. To obtain **1a** and **1b**, 1-(2-methoxyphenyl)piperazine and the appropriate N-(bromoalkyl)phthalimide were condensed in the presence of potassium carbonate in acetonitrile. **3a and 3b** were obtained when **1a** and **1b** was treated with isopropylamine (ratio 1: 7) in DMF at room temperature.

The yield of **3a** (25%) was low compared to **3b** (69%), whereas the reaction time of **3b** (56 h) almost doubled that of **3a** (32 h). The melting point of **3b** (120-122^{o}C) was lower than that of **3a** (152-153^{o}C). This shows that the length of the carbon chain has significant effect on the reaction rate, yield and melting point. The IR spectra revealed the presence of -NH peaks between 3737 and 3610 cm-^{1}, whereas the proton NMR signals demonstrated that the -CH- from the isopropylamine moiety appears as a multiplet and the -NH attached to the amine appeared as a doublet in the downfield region.

PHARMACOLOGY

Anti-inflammatory effect

The effect of the compounds on carrageenan-induced rat paw edema was more pronounced at the third hour of inflammation response, which corresponds to the phase of prostaglandin release (14). Compound **3a** (80 mg/kg) produced a significant ($p < 0.001$) inhibition in the edema level when compared to the control, after 3 h (Fig. 1) but at 20 mg/kg and 40 mg/kg there are still significant ($p < 0.05$) inhibitions in the edema level. As for compound **3b** (40 mg/kg) elicited a higher significant ($p < 0.001$) inhibition of the swelling caused by carrageenan at 3 h (Fig. 2), which was better than for indomethacin. The effect of **3b** on the inhibition of the edema level is more pronounced than that of **3a**.

From the results obtained in the anti-inflammatory and analgesic assays of compound **3a** and **3b,** it can be deduced that the length of the alkyl chain that separates the terminal amine from the phthalimido group has pronounced effect, which correlate with the work of previous researchers (13).

Analgesic effect

The *in vivo* analgesic activity of compound **3a** and **3b** was determined by mouse writhing assay which is a useful test for assessing mild analgesic NSAIDS and the results obtained are summarized in Table 1. Compound **3b** is the most potent and at 10 mg/kg it causes 71% inhibition that is much higher than that for acetylsalicylic acid, at a dose of 100 mg/kg and **3a** at a dose of 50 mg/kg. The results of the study show that at all doses used, the compounds significantly reduced acetic acid-induced writhes, which suggests that its analgesic effect could be peripherally mediated.

Statistical Analysis

All data were expressed as mean ± SEM and analyzed by the Student's t-test

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