ORIGINAL ARTICLE

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF CARBOXAMIDES

LUCKY O. OKUNROBO* AND CYRIL O. USIFOH

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

ABSTRACT

Some carboxamide derivatives with potential anti-inflammatory and analgesic properties were synthesized from the reaction of phthalimido alkyl acids with benzylamine at room temperature. All the compounds synthesized were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance (^{1}H and ^{13}C), Mass spectrophotometer, and elemental analyses. The carboxamides were evaluated pharmacologically for their *in vivo* anti-inflammatory and analgesic activities by carrageenan-induced rat paw oedema and acetic acid-induced writhing test respectively. All the investigated compounds exhibited significant anti-inflammatory activity in the range of 45 to 70% in comparison to control. They showed promising analgesic activity at the dose used. 3-Benzamido-propionic acid -2-(N-benzyl)-carboxamide (**4**) exhibited the highest anti-inflammatory and analgesic activities and the effects were dose-dependent.

Keywords: Benzylamine, phthalimido alkyl acids, carrageenan-induced oedema, mouse writhing test, carboxamides.

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 (Devillier, 2001). They are used widely in the treatment of pain and inflammation. These compounds, non selectively inhibit the two isoforms of the cyclooxygenase (COX-1 and COX-2) and thus prevent the metabolism of cellular arachidonic acid (AA) and the upregulation of prostaglandin formation, which otherwise lead to an increase of vascular permeability, oedema, hyperalgesia, pyrexia and inflammation. In addition to COX, the 5-lipooxygenase (5-LO) enzyme is another key enzyme, which is involved in the AA cascade. Leukotrienes produced through the 5-LO enzyme pathway, may also contribute to both inflammation and NSAIDs induced side effects (Almasirad *et al*., 2005).

Carboxamides and their derivatives have been synthesized and screened for various pharmacological properties including antitumor, antiviral, anti-inflammatory, analgesic activities among others (Gutshow *et al*., 2001; Collins *et al*., 2001; Raffa *et al*., 2002; Baldwin *et al*., 2005; Imamura *et al*., 2006).

Nakamura *et al* (1983) have shown in their studies that compounds containing carboxylic acid exhibit significant anti-inflammatory, analgesic, antipyretic and gastro-intestinal ulcerogenic activities. Our recent studies have showed that some phthalimides could be opened with sodium borohydride and 1-methylethylamine to obtain novel carboxamides with interesting anti-inflammatory and analgesic activities (Okunrobo *et al*., 2006a; Okunrobo *et al*., 2006b). The pharmacological importance of carboxamides and their derivatives encourage us to further investigate the products of the reaction of phthalimido alkyl acids with benzylamine and the carboxamides obtained were evaluated for analgesic and anti-inflammatory activities.

MATERIAL AND METHODS

Chemistry

Melting points measured on a Kofler hot stage apparatus and are 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.

General procedure

To a stirred solution of phthalimide derivatives in 10-20 ml of dimethylformamide or dichloromethane was added benzylamine dropwise and stirred at room temperature till TLC indicated disappearance of starting material. The reaction mixture was poured into 5 mL of 2M HCl and 30-40 mL of cold water and stirred for 5 min and then extracted with dichloromethane or ethylacetate. The organic phase was combined, washed with brine, dried over anhydrous Na_2SO_4. The solvent was removed *in vacuum*, and the

**Corresponding author*: Phone: +234-8034725416, e-mail: bricyedo@yahoo.com

residue was purified by column chromatography and recystallization in appropriate solvent to afford the desired products

***Benzamido-phenylmethylene-2-(N-benzyl) carboxamide*(3)**
To a solution of 4-phthalimidobutanoic acid (1.00 g, 4.29 mmol) in 10mL CH_2Cl_2 was added benzylamine (1.38 g, 12.87 mmol), stirred for 15 min and treated as in the general procedure to obtain a crude product and purified by recrystallisation from methanol to give benzamido-phenylmethylene-2-(N-benzyl) carboxamide. Yield :(1.19 g, 81%), mp: 170-171^oC, IR (KBr): 3247 (NH), 3077 (C-H), 1636 (C=O), 1560 (C=C), 797 (1,2-disubstitution) cm^{-1}; ^1H NMR (250MHz, DMSO-d_6) δ: 4.41-4.44 (d, 2H, J = 5.97 Hz, -CH_2 –Ar'), 7.24-7.40 (m, 5H, Ar'-H), 7.50-7.52 (dd, 4H, J = 2.0 Hz, Ar-H), 8.84 (t, 1H, J = 5.9 Hz, -NH); ^{13}C NMR (63MHz, DMSO-d_6) δ: 42.43(-CH_2-Ar') 126.61, 127.61, 128.15, 128.58, 129.34 (Ar'-C), 136.22,138.94, 139.40 (Ar-C), 168.17 (C=O); MS (m/z, 70 eV): 345(M^+ + 1, 2%), 327 (2), 238 (37), 237 (28), 209 (11), 180(20), 130 (7), 108 (16), 106 (12), 91(100), 77 (10), 65 (12); Elemental analysis: Calcd : C_{22} H_{20} N_2 O_2 (344.413): C, 76.72, H, 5.85, N, 8.13 Found: C, 76.55, H, 5.64, N, 8.02

***3-Benzamido-propionic acid-2-(N-benzyl)-carboxamide* (4)**
To a solution of 3-phthalimidopropionic acid (0.5 g, 2.28 mmol) in 20 mL of CH_2Cl_2 was added benzylamine (0.73 g, 6.84 mmol), stirred for 3 hours and treated as in the general procedure to give a crude product and purified by column chromatography (CH_2Cl_2 : EtOAc 5:1) and subsequent recrystallisation from methanol afforded white needle-like crystals of 3-benzamido-propionic acid -2-(N-benzyl)-carboxamide. Yield: (0.26 g, 35%) mp: 103-105^oC. IR (KBr): 3450 (NH), 3206 (OH), 2933 (C-H), 1777(COOH), 1712 (C=O), 1611 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.50-2.42 (q, 2H, J = 7.1 Hz, -CH_2-), 3.32-3.40 (t, 2H, J = 7.1 Hz, -CH_2-COOH), 4.43 (d, 2H, J = 5.9 Hz, -CH_2-Ar), 7.22-7.34 (m, 4H, Ar'-H), 7.43-7.49 (m, 4H, Ar-H), 8.24-8.28 (t, 1H, J = 5.0 Hz, NH), 8.74-8.78 (t, 1H, J = 5.0 Hz, NH); ^{13}C NMR (DMSO-d_6) δ: 33.57(-CH_2-), 35.25 (-CH_2-), 42.40 (-CH_2-Ar), 126.60, 127.09, 127.56, 128.13, 129.27(Ar'-C), 129.32, 135.94, 136.22, 139.39(Ar-C), 168.04 (C=O), 168.16 (C=O), 172.87 (COOH); MS m/z: 326 [M^+] (3%), 308 [M^+-H_2O] (5), 238 [M^+-88] (7), 237 [M^+-89] (48), 221(12), 220 (98), 202 (74), 180 (28), 160 (100), 106 (37), 104 (31), 77 (29), 51 (16).Elemental analysis: Calcd C_{18} H_{18} N_2 O_4 (326.352): C, 66.25, H, 5.56, N, 8.58 Found: C, 66.59, H, 5.76, N, 8.35

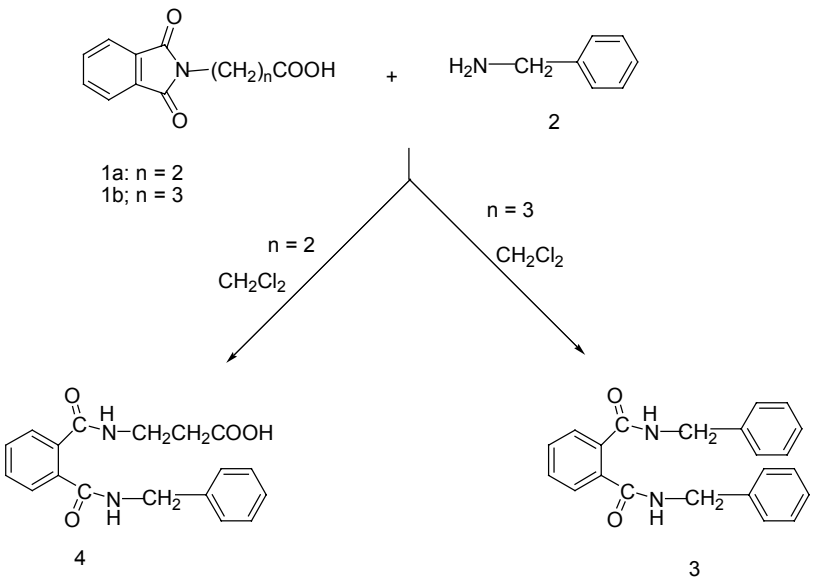
***4-Benzamido-butanoic acid -2-(N-benzyl)-carboxamide* (5)**
To a solution of 4-phthalimidobutanoic acid (0.50 g, 2.14 mmol) in 15 mL DMF was added benzylamine (0.69 g, 6.43 mmol), stirred for 7 hours and treated as in the general procedure to give a crude product which was recrystallised from methanol to give white crystals of 4-benzamidobutanoic acid -2-(N-benzyl)-carboxamide. Yield: (0.54 g, 70%). mp 162-164^oC. IR (KBr): 3643, 3416 (NH), 3208 (OH), 1771 (C=O), 1680 (C=O), 1516 (C-N), 1590 (C=C), 770 (1,2-disubstitution) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ: 1.68-1.73 (dd, 2H, J = 8.0 Hz, -CH_2-), 2.27-2.30 (t, 2H, J = 8.0Hz, CH_2COOH), 3.15-3.20 (q, 2H, J = 8.0Hz, HN-CH_2-), 4.40-4.42 (d, 2H, J = 8.0Hz, -CH_2-Ar), 7.21-7.32 (m, 5H, Ar'-H), 7.46-7.50 (m, 4H, Ar-H), 8.28-8.30(t, 1H, J = 8.0 Hz, NH), 8.78-8.80 (t, 1H, J = 8.0 Hz, NH); ^{13}C NMR (100MHz, DMSO-d_6) δ: 24.9 (CH_2), 31.7 (CH_2), 38.9 (CH_2), 42.9 (CH_2-Ar), 127.1, 127.7, 128.1, 128.7,129.0, 129.4 (Ar'-C), 129.7, 129.9, 136.4, 136.9, 139.9 (Ar-C), 168.7 (C=O), 168.8 (C=O), 174.9 (COOH). MS: m/z 341 (11%, M^+), 235(2), 234 (6); Elemental Analysis: Calcd. For $C_{19}H_{20}N_2O_4$ (340.379): C, 67.05; H, 5.92; N, 8.23. Found: C, 66.94; H, 5.61; N, 8.01.

Pharmacological evaluation
Swiss mice (18-23 g) and wistar rats (180-230 g) of either sex kept in the laboratory animal house 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.

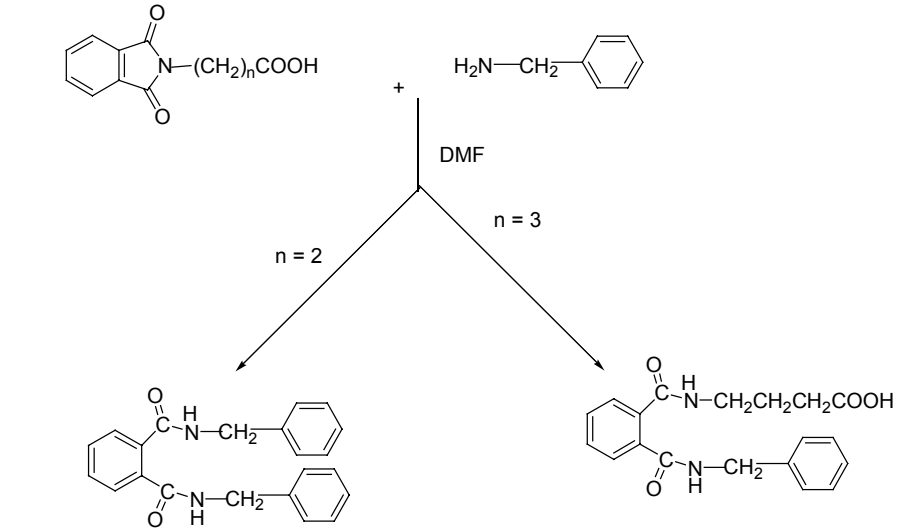
Anti-inflammatory activity
Anti-inflammatory activity was measured using carrageenan-induced rat paw oedema assay (Winter *et al*., 1962; Adeyemi *et al*., 2002). Group of 5 rats of both sexes (pregnant females excluded) were given a dose of a test compound. After one-hour 0.1 ml, 1% carrageenan suspension 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 four hours (Bamgbose and Noamesi 1981). Two groups of drug treated rats and one control group were 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.

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

Analgesic activity
Acetic acid induced writhing test (Koster *et al*., 1959; Adeyemi *et al*., 2004) was performed by an i.p. 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



Scheme 1



Scheme 2

gavage. Screening of analgesic activity was performed after p.o. administration of the test compounds at different dose level. After one hour 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 of 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 (Amir and Kumar, 2005) was expressed in term of % inhibition.

% Analgesic activity = (n – n'/n) x 100
(Where n = mean number of writhes of control group, n' = mean number of writhes of test group).

STATISTICAL ANALYSIS

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 the test compounds.

RESULTS

The reactions of 3-phthalimidopropionic acid **1a** and 4-phthalimidobutanoic acid **1b** with excess benzylamine in dichloromethane (scheme 1) afforded 3-benzamido-propionic acid -2-(*N*-benzyl)-carboxamide (**4**) and benzamido-phenylmethylene-2-(*N*-benzyl) carboxamide (**3**) respectively. The reaction of **1b** with benzylamine gave a dimmer, which revealed that the benzylamine completely

Table 1: Effect of the test compounds on acetic acid induced writhing in mice

Compounds	Doses in mg/kg (p.o)	Numbers of writhing (per 20 min)	% Inhibition
Control 10% Tween 80	0.2ml	56.86 ± 4.21	-
Acetylsalicylic acid	100	22.50 ± 3.07 [b]	60.43
Indomethacin	10	14.80 ± 4.95 [b]	73.97
3	20	38.25 ± 3.84 [a]	32.73
	40	25.25 ± 2.29 [b]	55.59
4	10	36.25 ± 1.75 [a]	36.25
	20	15.00 ± 2.55 [b]	73.62
5	10	49.33 ± 5.48 [a]	13.24
	20	21.50 ± 6.91 [b]	62.19

Values are mean ± S.E.M [a] P< 0.05, [b] P< 0.001, significantly different from control, Paired t- test (n = 5-8), p.o = per oral

Table 2: Effect of the test compounds on the carrageenan-induced rat paw oedema

Compounds	Doses mg/kg (p.o)	Change in paw oedema mean ± SEM in mm	% oedema inhibition relative to control at the 4th hour
Control 10% Tween 80	0.3ml	4.51 ± 1.35	-
Indomethacin	10	1.42 ± 0.36**	68.51
3	20	2.50 ± 0.35*	44.56
	40	2.17 ± 2.45**	51.88
	80	1.67 ± 0.58**	62.97
4	20	2.38 ± 0.24*	47.23
	40	1.63 ± 0.38**	63.86
	80	1.35 ± 0.38**	70.07
5	20	3.13 ± 0.31*	30.71
	40	2.25 ± 1.49**	50.11
	80	1.50 ± 0.34**	66.74

Values are mean ± S.E.M * P< 0.05, ** P< 0.001, significantly different from control, Paired t- test (n = 5-8), p.o = per oral

replaces the aliphatic carboxylic acid group in **1b**, which was not observed when **1a** was used. However when **1a** and **1b** were treated with excess benzylamine in dimethylformamide (scheme 2) it afforded benzamido-phenylmethylene-2-(*N*-benzyl) carboxamide (**3**) and 4-benzamido- butanoic acid -2-(*N*-benzyl)-carboxamide (**5**) respectively. Furthermore when equimolar ratio of **1a** or **1b** to benzylamine in either solvent medium was used no significant products were formed.

DISCUSSION

The infrared spectra of the compounds synthesized revealed that –NH- appears at between 3643-3206 cm^{-1}. Compound **3** revealed the presences of only one –NH-, one C=O, and one $–CH_2$- signal, though we have two of these functional groups present in compound **3.** These show that compound **3** is a dimmer, but compounds **4** and **5** revealed the presence of two different –NH- in different chemical environment. The proton nuclear magnetic resonance of compound **3** also confirmed the dimmer nature. The –NH- appeared at 8.84 ppm as a triplet with a coupling constant of 5.96 Hz due to the presence of two neighbouring proton of $–CH_2$- whereas the $–CH_2$- from the benzyl group appears at 4.41- 4.44 ppm as a doublet with a coupling constant of 5.97 Hz.

Carrageenan-induced oedema of rat paw is used widely as a working model of inflammation in the search for new anti-inflammatory agents (Manueli *et al*., 1994) and appeared to be the basis for the discovery of indomethacin, an anti-inflammatory drug (Winter *et al*., 1963). The oedema, which develops in rat paw after carrageenan injection, is a biphasic event (Vinegar *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin while the oedema maintained between the first and second phase is due to kinin and the second phase attributed to prostaglandin (DiRosa and Willoughby, 1971). All the mediators appear to be dependent upon an intact complement system for their activation and release (Giroud and Willoughby, 1970). All the compounds **3, 4** and **5** tested show significant activity and had a dose-dependent effect. However, **4** showed the

highest activity in comparison to control. This shows that compounds containing carboxylic acid could be employed as anti-inflammatory agents.

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. Compound **4** shows the highest activity at 20 mg/kg compared to the other compounds and acetylsalicylic acid, nonetheless the activity is comparable to that of indomethacin. Compound **3** showed the least activity.

ACKNOWLEDGEMENT

The authors appreciate the assistance of Prof. G.K.E Scriba, University of Jena, Germany in running some of the spectra and University of Benin, Benin city for the URPC grant (REG/ER/URPC.SS/S2002/09) given to Okunrobo L.O.

REFERENCES

Adeyemi OO, Okpo SO and Ogunti OO (2002). Analgesic and anti-inflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia*. **73**: 375-380.

Adeyemi OO, Okpo SO and Okpaka O (2004). The Analgesic effect of the methanolic extract of *Acanthus montanus*. *J. Ethnopharmacol*. **90**: 45-48.

Almasirad A, Tajik M, Bakhtiari D, Shafiee A, Abdollahi M, Zamani MJ, Khorasani R and Esmaily H (2005). Synthesis and analgesic activity of arylhydrazone derivatives of mefenamic acid. *J. Pharm. Pharmaceut. Sci*., **8**(3): 419-425.

Amir M and Kumar S (2005). Anti-inflammatory and gastro sparing activity of some new Indomethacin derivatives. *Arch.Pharm. Chem. Life Sci*., **338**: 24-31.

Baldwin J, Michnoff CH, Malmquist NA, White J, Roth MG, Rathod PK and Phillips MA (2005). High-throughput screening for potent and selective inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase. *J. Biol. Chem*., **280**: 21847-21453.

Bamgbose SOA and Noamesi BK (1981). Studies on cryptolepine II: Inhibition of carrageenan induced oedema by cryptolepine. *Planta Medica*., **41**: 392-396.

Collins X, Robert JM, Wielgosz G, LeBaut G, Bobin-Dubigeon C, Grimaud N and Petit JY (2001). New anti-inflammatory N-pyrindyl(Alkyl)phthalimides acting as tumor necrosis factor-alpha production inhibitors. *Eur. J. Med. Chem*., **36**: 639-649.

Devillier P (2001). Pharmacology of non-steroidal anti-inflammatory drugs and ENT pathology. *Presse Med*., **30**: 70-79.

DiRosa M and Willoughby D A (1971). Screens for anti-Inflammatory drugs. *J. Pharm. Pharmacol*., **23**: 297-298.

Duffy JC, Dearden JC and Rostron C (2001). Design, Synthesis and biological testing of a novel series of anti-inflammatory drugs. *J. Pharm. Pharmacol*., **53**: 1505-1514.

Giroud JP and Willoughby DA (1970). The interrelations of complement and a prostaglandin-like substance in acute inflammation. *J. Path*., **101**: 241.

Gutshow M, Hecher AT, Thiele A, Hauschildt S and Eger K (2001). Aza analogues of thalidomide: Synthesis and evaluation as inhibitors of tumor necrosis factor-alpha production *in vitro*. *Bioorg. Med. Chem*., **9**: 1059-1065.

Imamura S, Ichikawa T, Niishikawa Y, Kanzaki N, Takashima Baba M and Sugihara Y (2006). Discovery of a piperidine-4-carboxamide CCR5 antagonist as potent anti-HIV-1 activity. *J. Med. Chem*., **49**(9): 2784-2793.

Koster R, Anderson M and Debeer E.J (1959). Acetic acid analgesic screening. *Federation Proceedings,* **18**: 418-420.

Manueli VEF, Diaz G, Gonzalez A and Bermejo J (1994). Antinociceptive, Anti-inflammatory and antipyretic effects of Lapidin, a bicyclic sesqiterpene. *Planta Medica*, **60**: 395-399.

Nakamura H, Yokoyama Y, Motoyoshi S, Ishii K, Imazu C, Seto Y, Kadokawa T and Shimizu M (1983). The pharmacological profile of 2-(8-methyl-10, 11-oxodibenz[b,f]oxepin-2-yl)propionic acid (AD-1590), a new nonsteroidal anti-inflammatory agent with potent antipyretic activity. *Arzneimittelforschung,* **33**(11): 1555-1569.

Okunrobo LO, Usifoh CO and Scriba GKE (2006a). Synthesis and pharmacological evaluation of 2-hydroxymethylbenzamides as anti-inflammatory and analgesic agents. *Acta Pol. Pharm.,* **63**: 25-31.

Okunrobo LO, Usifoh CO and Okpo SO (2006b). Reactions of phthalimides with 1-methylethylamine: analgesic and anti-inflammatory properties of resulting carboxamides. *Pak. J. Pharm. Sci.*, **19**(1): 34-38.

Raffa D, Daidone G, Plescia F, Schillaci D, Maggio B and Torta L (2002). Synthesis and antifungal activity of new N-(1-phenyl-4-carbetoxypyrazol-5-yl)-, N-(indazol-3-yl)- and N-(indazol-5-yl)-2-iodobenzamides. *Farmaco,* **5**: 183-187.

Vinegar R., Schreiber W and Hugo R (1969). Biphasic development of carrageenan oedema in rats. *J. Pharmacol. Exp. Ther*. **166**: 96-100.

Winter CA, Risley EA and Nuss GW (1962). Carrageenan-induced oedema in the hind paw of rat as an assay for anti-inflammatory activity. *Proc. Soc. Exp. Biol. Ther*. **111**: 544-547.

Winter CA, Risley EA and Nuss GW (1963). Anti-inflammatory and anti-pyretic activities of indomethacin, 1- (p-Chlorobenzyl) -5-methoxy-2-Methylindole 3-acetic acid. *J. Pharmac. Exp. Ther*., **141**: 369.

Received: 19-09-2006– Accepted: 10-10-2006