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Synthesis and anti inflammatory activity of 2-substituted-[(n, n-disubstituted) - 1, 3-benzoxazol]-5-carboxamides

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ABSTRACT

A series of 2-substituted-[(N,N-disubstituted)1,3-benzoxazol]-5-carboxamides derivatives were synthesized by reaction of 2-substituted-5-carbomethoxy benzoxazole with different secondary amines. The newly synthesized compounds were characterized on the basis of spectral (FT-IR, ^{1}H NMR) analysis. All these compounds were screened for anti-inflammatory activity using carrageenan induced rat paw edema method. All of these compounds exhibited significant activity. Among the tested compounds, Va, Vb and Ve were considered to have potent anti inflammatory activity and was comparable with standard.

KEYWORDS

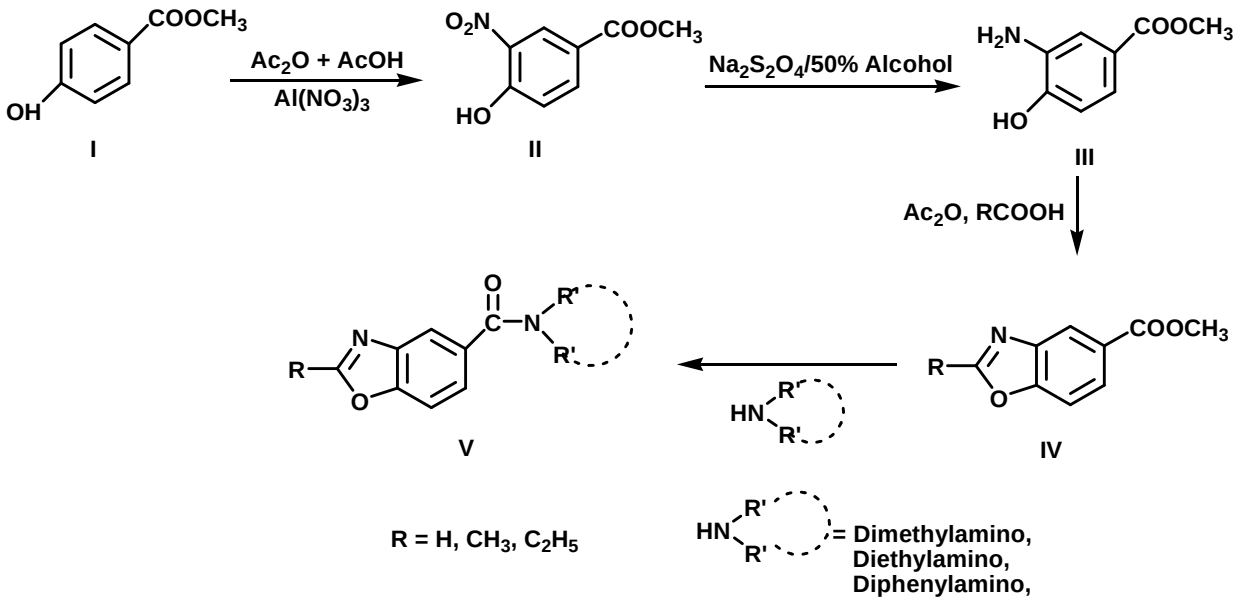
Benzoxazole;
Anti-inflammatory activity;
Carrageenana.

INTRODUCTION

The substituted benzoxazoles have attracted much attention due to their prominent utilization as anti-inflammatory[1], antiviral[2], antifungal[3], anti-bacterial[4], anticancer[5], antitubercular[6], anticonvulsant[7] and hypoglycemic[8] activity probably resulting from its planar and compact structure. Most of the work cited in the literature advocates benzoxazole, a heterocyclic compound as a promising lead for analgesic and anti-inflammatory property[9,10]. It has been reported that 2-oxo-3H-benzoxazoles bearing N-alkyl, N-acyl, N-diaminoalkyl and 6-acyl substituents were reported to have higher analgesic and anti-inflammatory activity[11,12]. Studies showed that these benzoxazole moieties exerted their invivo activity by inhibiting the synthesis of prostaglandin E_2[13]. Therefore, in view of the varied biological and pharmacological properties of benzoxazoles, it has been considered as prime importance to synthesis different 2-substituted-(N,N-disubstituted)-5-carboxamide benzoxazole derivatives with a view to screen the products for anti-inflammatory effect by carrageenan induced rat paw edema method.

EXPERIMENTAL

Melting points were determined in open capillaries, using Toshniwal melting point apparatus,expressed in ^oC and are uncorrected. The IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series,FT-IR spectrometer using KBr disc.^{1}H NMR were recorded on a Avance-300 MHz instrument using TMS as an internal standard(chemical shifts in δ,ppm).The physical data of the synthesized compounds are given in TABLE 1



Scheme

Physical data of titled compounds has been presented in TABLE 1

TABLE 1 : Physical data of 2-substituted-[(N, N-disubstituted) - 1, 3-benzoxazol]-5-carboxamides (V)

S. No	Compound	R	—N(R')(R')	Molecular Formula	M.P. (°C)	Yield (%)
1.	V a	H	Dimethylamino	$C_{10}H_{10}O_2N_2$	220-24	85
2.	V b	CH_3	Dimethylamino	$C_{11}H_{12}O_2N_2$	200-04	80
3.	V c	C_2H_5	Dimethylamino	$C_{12}H_{14}O_2N_2$	160-62	65
4.	V d	H	Diethylamino	$C_{12}H_{14}O_2N_2$	210-12	53
5.	V e	CH_3	Diethylamino	$C_{13}H_{16}O_2N_2$	174-76	75
6.	Vf	C_2H_5	Diethylamino	$C_{14}H_{18}O_2N_2$	110-13	55
7.	Vg	H	Diphenylamino	$C_{20}H_{14}O_2N_2$	185-87	85
8.	Vh	CH_3	Diphenylamino	$C_{21}H_{16}O_2N_2$	155-56	85
9.	Vi	C_2H_2	Diphenylamino	$C_{22}H_{18}O_2N_2$	145-48	60

Synthesis of 4-carbomethoxy-2-nitrophenol (II)

To a solution of aluminium nitrate (40 g) in acetic acid - acetic anhydride (1:1) mixture (160 ml) was added an appropriate phenol (I, 40g) in small portions, while cooling and shaking, occasionally. The reaction mixture was left at room temperature for 1.5 hours while shaking the contents, intermittently to complete the nitration. The resulting brown solution was diluted with ice-cold water (500 ml) and acidified with concentrated nitric acid (40 ml) to get a bulky, yellow precipitate. It was filtered washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline solid (44 g, 85%), m.p. 70°C.

Synthesis of 4-carbomethoxy-2-aminophenol (III)

4-Carbomethoxy-2-nitrophenol (II, 10 g) was dissolved in boiling alcohol (50%, 100 ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colorless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with ice-cold water. The resulting colorless, shiny product was filtered, washed with cold water and dried. Its purification was effected by recrystallisation from benzene to get colorless, shiny scales (5.1 g; 60%), m.p. 140°C

Synthesis of 2-substituted-5-carbomethoxy benzoxazole (IV)

4-Carbomethoxy-2-aminophenol (III, 0.01 mol) has been refluxed with an appropriate aliphatic acid in excess viz. formic acid, acetic acid and propionic acid for 2 hours. The reaction mixture has been cooled and poured in to crushed ice. The product obtained is purified by recrystallization from methanol has resulted a crystalline white solid, (m.p. 162° C, yield 70 %).

IV) Synthesis of 2-substituted-[(N,N-disubstituted)-1,3-benzoxazol]-5-carboxamides (V)

A mixture of an appropriate 2-substituted-5-carbomethoxy benzoxazole (IV, 0.01mol) and appropriate secondary amines was heated under reflux for 4 hrs in the presence of absolute alcohol (25ml). The

product separated was filtered and washed with small portions of crushed ice repeatedly and filtered. The product was purified by recrystallization from suitable solvent(s).

The synthesized compounds were purified by column chromatography and were characterized by IR and ^{1}H NMR (TABLE 2).

TABLE 2 : Spectral data of compounds (V_a-V_e)

Compound	IR(KBr),cm^{-1}	^{1}H NMR(DMSO-d_6), δ ppm
V_a	2924 (C-H str), 1691 (C=O), 1604 (C=C),1480 (C=C,Ar) 1462 (C=N) and 1114(ether, C-O-C).	6.4-7.2 (m, 4H, Ar-H), 3.4 [s, 6H, {N-(CH_3)$_2$}].
V_b	3014 (CH str), 1688 (C=O), 1545, (C=N, cyclic), 1488 (C=C, Ar),	6.8-7.8 (m, 4H,Ar-H), 2.63(s,3H,CH_3), 3.4 [s, 6H, {N-(CH_3)$_2$}]
V_c	2935 (CH str), 1601 (C=O), 1563(C=N, cyclic), 1488 (C=C, Ar),	6.7-7.8 (m, 4H,Ar-H), 3.4 [s, 6H, {N-(CH_3)$_2$}], 2.5 (m,2H,CH_2), 2.2 (m,3H,CH3),
V_d	2953 (C-H stretching), 1691 (C=O), 1604 (C=C), 1438 (C=N) and 1114 (ether, C-O-C)	6.4-7.2 (m, 4H, 4H, benzoxazole, 3.4 [s, 4H, {N-(CH_2)$_2$}] and 2.3 {s, 6H, (CH_3)$_2$}
V_e	2917 (CH str), 1668 (C=O), 1530 (C=N, cyclic), 1485 (C=C, Ar), 1332 (C=S), 1021(C-O-C),	6.4-7.2 (m, 4H, 4H, benzoxazole, 3.4 [s, 4H, {N-(CH_2)$_2$}] , 3.1(s,3H,CH_3) and 2.3 {s, 6H, (CH_3)$_2$}

Screening for anti-inflammatory activity by rat hind paw edema method

Animals

Male Wister albino rats weighing 200-230 gm were used for the experiment. The animals were housed in colony cages (six rats each), maintained on a standard pellet diet and water ad libitum and left for 2 days for acclimatization before the experimental sessions. The food was withdrawn on the day before the experiment, but they were allowed free access to water. The protocol of the present study was approved by the Institutional Animal Ethical Committee.

Test Samples

The suspension of all test samples were prepared using a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC) and were administered orally to the animals. All the test compounds were tested at a dose of 100 mg/kg body weight

Carrageenan induced rat hind paw edema method

The normal paw volumes of all the rats were measured initially and were divided into seven groups of six animals each and were treated with the vehicle as control (0.5% sodium CMC), standard diclofenac sodium (20 mg/kg) and five test compound (V a-Ve, 100 mg/kg) respectively. Carrageenan (0.1 ml of a 1% suspension in saline) was injected into the sub plantar region of the right hind paw of each rat. The vehicle, Standard and test compounds were administered 30 min prior to the injection of Carrageenan. The swelling (paw volumes) produced after injection of the phlogistic agent was measured in all the rats at 1, 2, 3 and 4 hr after Carrageenan treatment by using plethysmometer[14]. A significant reduction in the paw volume compared to vehicle treated control animals was considered an inflammatory response.

$$\% \text{ Inhibition} = [(V_T - V_0) \text{ control} - (V_T - V_0) \text{ treated groups}] / (V_T - V_0) \text{ control} * 100$$

V_0= paw volume of the rat before administration of Carrageenan

V_T= paw volume of the rat after administration of Carrageenan at different time intervals

Statistical Analysis

All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. *P*-values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The preliminary studies on pharmacological activity of the new benzoxazole derivatives have generated some interesting data. Here, an attempt has been made to infer the results obtained from the study on anti-inflammatory activity. The anti-inflammatory activity of five test compounds has been evaluated against Carrageenan induced paw edema in rats and the results are presented in TABLE 3 using Diclofenac as standard.

TABLE 3 : Effect of 2- substituted-[(N, N- disubstituted)-1, 3-benzoxazol] - 5-carboxamides on the paw edema test in rats

S. No	Compound	Paw oedema volume in ml (% Inhibition)			
		1 hr	2 hr	3 hr	4 hr
1	Control	0.36±0.10	0.70±0.15	0.73±0.14	0.77±0.12
2	Diclofenac	0.23±0.04[a] (36.11)	0.29±0.02 [a] (68.57)	0.31±0.006 [a] (51.50)	0.47±0.05 [a] (38.96)
3	V a	0.18±0.05[a] (50.00)	0.30±0.04 [a] (67.14)	0.15±0.05 [a] (61.69)	0.35±0.04 [a] (54.55)
4	V b	0.27±0.07 [a] (25.00)	0.29±0.03 [a] (58.57)	0.35±0.09 [a] (33.96)	0. 60±0.10 [a] (22.07)
5	V c	0.29±0.10 [a] (19.44)	0.45±0.14 [a] (35.71)	0.32±0.12 [a] (39.62)	0.52±0.09 [a] (32.47)
6	V d	0.31±0.06 [a] (13.89)	0.49±0.08 [a] (30.00)	0.32±0.12 [a] (40.52)	0.61±0.10 [a] (20.78)
7	V e	0.19±0.03 [a] (47.22)	0.24±0.04 [a] (65.71)	0.13±0.06 [a] (77.47)	0.35±0.03 [a] (54.55)

All the values are expressed as mean±SD, (n=6); [a] P<0.005 vs Control

Based on these results, all the synthesized benzoxazole derivatives exhibited a significant anti-inflammatory activity against Carrageenan induced paw edema. Among the tested compounds, Va, Vb and Ve were considered to have potent anti inflammatory activity and was comparable with standard anti inflammatory drug, diclofenac.

Carrageenan-induced rat paw oedema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-oedematous effect of natural products[15]. Development of oedema in the paw of the rat after injection of Carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of oedema is due to the release of prostaglandins, protease and liposome[16,17]. Diclofenac sodium, a COX-inhibitor at the dose of 20 mg/kg, p.o. significantly reduced the paw oedema. This indicates action against release of histamine, serotonin and kinins in early phase, while later phases are suspected to be arachidonate metabolites producing an oedema dependent on mobilization of neutrophils[17]. The reduction in paw volume after administration of all the 2- substituted-[(N, N- disubstituted)-1, 3-benzoxazol] - 5-carboxamides indicated the anti-inflammatory activity of the test compounds which is might be due to the inhibition of either release or synthesis of prostaglandins, histamine and serotonin.

In conclusion, all the new 2-substituted benzoxazole derivatives showed promising anti-inflammatory activity when compared to the standard drug. It has been felt necessary, from the results of the present preliminary investigations that there is a need for further advanced studies, at least on the few of the test compounds which are found to be superior.

REFERENCES

[1] S.M.Sondhi, S.Singh, A.Kumar, O.Lozach; Bioorg.Med.Chem., **14**, 3758 **(2006)**.

[2] C.Burkhloder, G.Laumond, M.Medebielle; J.Fluor.Chem., **126**, 535 **(2005)**.

[3] K.Cermakova, A.Tomeckova, J.Vinsova; Bioorg.Med.Chem., **14**, 5850 **(2006)**.

[4] I.Yalcin, E.Aki-Sener, N.Altanlar; Arch.Pharm.Med.Chem., **337**, 402 **(2004)**.

[5] E.Aki-Sener, H.Lage, I.Yalcin; Int.J.Cancer, **119**, 213 **(2006)**.

[6] I.I.Rodriguez, A.D.Rodriguez; J.Nat.Prod., **66**, 855 **(2003)**.

[7] S.Spampinato, H.Ucar, S.Cacciaguerra; Eur.J.Pharmacol., **355**, 267 **(1997)**.

[8] Kenji Arakawa, Masanori Inamasu, Mamoru Matsumoto, Kunihito Okumura, Kosuke Yasuda, Hidenori Akatsuka, Saburo Kawanami, Akishige Watanabe, Koichi Homma, Yutaka Saiga, Masakatsu Ozeki, Ikuo Iuima; Chem.Pharm.Bull., **45**, 12 **(1997)**.

[9] D.S.Doğruer, S.Ünlü, M.F.Sahin, E.Yēsilada; Farmaco, **53**, 80 **(1998)**.

[10] J.Mercier, C.Lespagnol, M.R.Sestier; Bull.Soc.Lille., **35**, **(1953)**.

[11] S.Unlü, H.Erdogan, R.Sunal, B.Gümüş el; J.Fac.Pharm.Gazi, **9**, 75 **(1992)**.

[12] G.Pilli, F.Özkanl, C.S.afak; H.Erdogan, S.Ünlü, B.Gümüş el, R.Demirdamar; Pharmazie, **49**, 63 **(1994)**.

[13] C.Safak, H.Erdoğan, E.Palaska, R.Sunal, S.Duru; J.Med.Chem., **35**, 1296 **(1992)**.

[14] R.A.Turner; Screening methods in Pharmacology, **27**, 22 **(1995)**.

[15] R.Vinegar, W.Schreiber, R.Hugo; J.Pharmacol.Exp.Ther., **166**, 96 **(1969)**.

[16] P.Crunkhon, S.E.R.Meacock; Brit.J.Pharmacol., **42**, 392 **(1971)**.

[17] M.J.Just, M.C.Recio, R.M.Giner, M.J.Cullar, S.Manez, A.R.Bilia; Planta Medica, **64**, 404 **(1998)**.