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Drug-induced modifications of the immune response 17. 2-Benzoxazolecarboxamide derivatives

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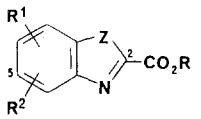
Introduction

In the late 1960's cromolyn sodium (sodium cromoglycate) was introduced into the clinic for the prophylactic treatment of allergic disorders such as asthma, rhinitis and conjunctivitis [1–4]. Because of its rapid elimination from the body, cromolyn sodium is effective only when administered by inhalation. In recent years, research efforts have been directed towards the synthesis of topically effective agents having longer duration of action and a greater potency than cromolyn sodium. As a result, two novel drugs, nedocromil sodium and minocromil are currently undergoing therapeutic evaluation [5–8].

In an earlier report [9], we disclosed the synthesis and antiallergic activity of a series of novel esters of substituted benzoxa(thia)zole-2-carboxylic acids **(1)** [10]. One analogue, RG 2871 (REV 2871, CHBZ) (**1**; R = CH_2CH_2-



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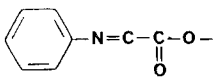
OC_2H_5, R^1 = H, R^2 = 5–Cl, Z = O) is currently undergoing clinical trials as a novel orally active antiallergic agent. RG 2871 and its putative metabolite RG 3579-Z (REV 3579-Z) (**1**; R = H (as the Na salt), R^1 = H, R^2 = R–Cl, Z = O) were shown to be potent inhibitors of (a) passive cutaneous anaphylaxis (PCA) in rats (ED_{50} = 12 mg/kg), (b) the immunologic and non-immunologic release of histamine from rat mast cells (ID_{50} = 2–20 μM) [11]. Data from these and other assays have demonstrated that, overall, RG 2871 possessed a different mechanism of action as an inhibitor of mediator release from that of cromolyn sodium and proxicromil [11, 12].

We have postulated [10] that unit **2**, a building block of 1 as well as some other structurally similar molecules [13, 14] is an important feature in conferring of antiallergic activity *via* the inhibition of mediator release.



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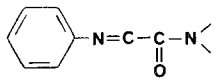

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It was further interest to determine whether replacing the 2-ester group of **2** with an amide function (such as in **3**) will have any effect on the antiallergic activity of **1**. The present communication discusses some of our studies in this direction, namely the synthesis and antiallergic activity of a number of 2-benzoxazolecarboxamide derivatives. Previous reports have indicated that various 2-benzoxazolecarboxamides exhibited antitubercular [15, 16], CNS [17] and anti-inflammatory [18] activities. In addition, some analogues were found to be active as plant growth regulators [19] and herbicides [20].

Chemistry and Pharmacology

The synthesis of the title 2-benzoxazolecarboxamides **(5)** was accomplished by conversion of 3-chloro-2*H*-1,4-benzoxazin-2-one **(4)** [10] in the presence of appropriate amines [21] (Scheme 1). Treatment of **5** with phosphorus pentasulfide-pyridine afforded the corresponding 2-benzoxazolethiocarboxamide analogues **6**. The reaction of benzothiazine-2,3-dione **(7)** with aniline in glacial acetic acid provided the 2-benzothiazolecarboxanilide **(8)** (Scheme 1).

The ring contraction of 3-chloro-2*H*-1,4-benzoxazin-2-one **(4)** to the 2-benzoxazolecarboxamides **5** proceeded by a mechanism similar to the one described previously [24] for 3-bromocoumarine.

The antiallergic activity of the title 2-benzoxazolecarboxamides **5** and related derivatives **6** and **8** was eva-

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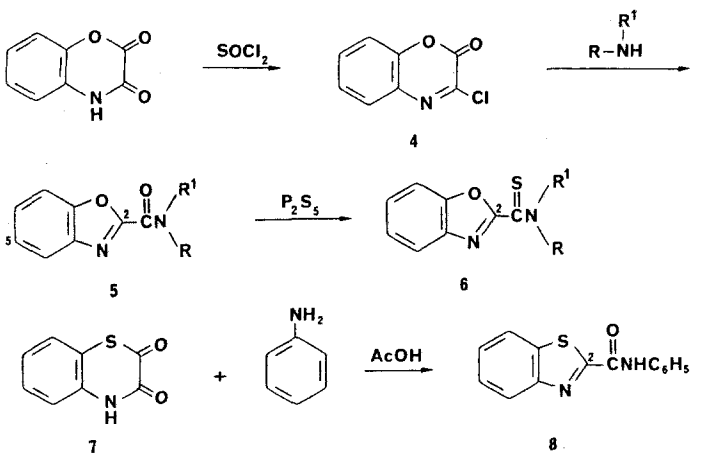
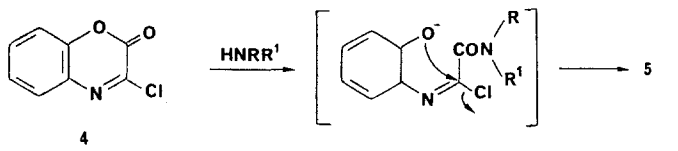


Scheme 1.

luated in the passive cutaneous anaphylaxis (PCA) assay in rats (Table I). As seen in Table I, the *N,N*-dimethyl-2-benzoxazolecarboxamide **(5d)** demonstrated the most potent activity when administered at a dose of 50 mg/kg



by the intraperitoneal (i.p.) route. The *N*-monomethyl **(5b)** and the unsubstituted **(5a)** carboxamides showed a decreased potency as did the ones having *N-tert*-butyl **(5c)** and *N*-phenyl substituents. Substituting the carbonyl group with a thiocarbonyl function (**6a** and **6b**) resulted also in a diminished potency. When compared to the corresponding 2-benzoxazolecarboxamide analogue **5e,** the thio derivative **8** exerted a similar activity.

Overall, when evaluated in the PCA assay, the antiallergic activity of esters **1** was significantly superior to that of the 2-benzoxazolecarboxamides **5.**

In addition to their antiallergic activity, the *N*-alkyl derivatives **5b** and **5d** elicited moderate anti-inflammatory activity in the carrageenin-induced rat paw edema test, while analogues **5a** and **5e** were found to be essentially inactive. The lack of any meaningful activity of **5** in the dermal vascular permeability assay in rats should rule out the inhibition of action of the mediators of the small blood vessels as a viable mechanism for antiallergic activity.

The experimental results of the present study lend further credence to the postulation [10, 13, 14] that units **2** and **3** act as potential pharmacophores for antiallergic activity.

Experimental Protocols

All melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained as KBr discs on a Perkin–Elmer 727 B spectrophotometer. Proton nuclear magnetic resonance spectra were recorded on a Varian EM 306 instrument using tetramethylsilane as internal standard. Mass spectra were taken on a Varian MAT 112 instrument. All spectra were consistent with the assigned structures.

2-Benzoxazolecarboxamide ***(5a)***

10 ml of NH_3 was dried over sodium and evaporated into a Dewar flask, then condensed into a 100-ml flask containing 50 ml of anhydrous tetrahydrofuran (freshly dried over lithium aluminum hydride) submurged in a dry-ice/acetone bath. The cold NH_3 solution was added portionwise at −30 to −10°C, to a solution of **4** (12.9 g, 71 mmol) in 100 ml of anhydrous tetrahydrofuran cooled in dry-ice/acetone bath. The reaction mixture was stirred for 1 h, then the temperature was allowed to rise to

Table I. 2-Benzoxazolecarboxamides **(5)** and related compounds: structures and biological activity.

Compd	R	R^1	PCA assay (% inhibition)	Carrageenin-induced rat paw edema (% inhibition)	Dermal vascular permeability assay (% inhibition)
5a	H	H	43.0[a]	15.9[b]	3.2
5b	H	CH_3	69.0[a]	25.9[a,c]	–
5c	H	C_4H_9-*tert*	22.3[a]	–	–
5d	CH_3	CH_3	81.9[a]	26.8[a,b]	17.5
5e	H	C_2H_5	18.8	8.5[d]	–
6a	H	CH_3	14.6	–	–
6b	CH_3	CH_3	18.6	–	–
8			17.2	–	–
Cromolyn sodium			51.0[a,e]	–	–
Indomethacin			–	52.0[a,f]	–
Cyproheptadine			–	–	48.0[a,g]

[a]Treated groups differed from controls at $P < 0.05$ (unpaired Student's *t*-test); [b]40 mg/kg, p.o.; [c]60 mg/kg, *p.o.*; [d]30 mg/kg, i.p.; [e]6 mg/kg, i.p.; [f]4 mg/kg, p.o.; [g]1 mg/kg, i.p.

20°C. After stirring for 1 h at 20°C, the reaction mixture was filtered to remove NH_4Cl and concentrated under reduce pressure to yield 11.1 g of an orange-colored solid which was chromatographed on silica gel leaving 6.97 g (60%) of **5a**, mp 176–177°C (chloroform) (lit [21] mp 176–178°C).

N-*Methyl-2-benzoxazolecarboxamide* ***(5b)***
To a solution of **4** (8.5 g, 46.8 mmol) in 80 ml of anhydrous tetrahydrofuran was added dropwise 1.45 g (46.5 mmol) of methylamine at −10 to 5°C. The reaction mixture was allowed to warm to ambient temperature and the solvent was evaporated under reduced pressure leaving a brown-colored solid which was purified by chromatography on silica gel leaving 6.8 g (83%) of pure **5b**, mp 119–121°C (water) (lit [21] mp 119–121°C).

N-tert-*Butyl-2-benzoxazolecarboxamide* ***(5c)***
Compound **5c** was obtained by a procedure similar to that described for **5b** starting from **4** (8.15 g, 45 mmol) and *tert*-butylamine (6.6 g, 90 mol) and purified by chromatography on silica gel using chloroform as the eluent. Yield: 3.23 g (33%). mp 97–98°C (ether-hexane). Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C: 66.03; H: 6.47; N: 12.84; found: C: 65.98; H: 6.55; N: 12.83.

In addition to **5c**, a small amount (0.5 g, 5%) of 3-*tert*-butylamino-2*H*-1,4-benzoxazin-2-one was also obtained following the chromatography of the crude reaction product.

N,N-*Dimethyl-2-benzoxazolecarboxamide* ***(5d)***
To a solution of **4** (13.52 (74.5 mmol) in 120 ml of anhydrous tetrahydrofuran at −30°C was added dropwise from a Dewar condenser 3.35 g (74.5 mmol) of dimethylamine. The reaction mixture was allowed to warm to ambient temperature and filtered. The concentrated filtrate was chromatographed on silica gel using a mixture of chloroform-methanol as eluent, to give 14.1 g (99%) of **5d**, mp 80–81°C (ether-hexane) (lit [21] mp 76–78°C).

N-*Phenyl-2-benzoxazolecarboxamide* ***(5e)***
Compound **4** (7.27 g, 0.04 mol) was dissolved in 30 ml freshly distilled (over lithium aluminum hydride) tetrahydrofuran. Aniline (5.60 g, 0.06 mol) was added and the reaction mixture was heated to 55–60°C for 3.5 h. Upon cooling the solid precipitate was filtered off giving 3.60 g (38%) of 3-phenylamino-1,4-benzoxazin-2-one, mp 198–200°C (benzene-ether, 1:1). The filtrate was concentrated to a solid which was stirred in hot methanol for 15 min, then the insoluble material was filtered off and the solvent evaporated under reduced pressure yielding 2.6 g (27%) of **5e** after recrystallization from benzene-hexane (3:4), mp 154.5–157°C (lit [21] mp 155–157°C).

N-*Methyl-2-benzoxazolethiocarboxamide* ***(6a)***
A mixture of **5b** (6.8 g, 38.6 mmol) and phosphorus pentasulfide (10.0 g, 46 mmol) in 100 ml of pyridine was refluxed for 90 min then cooled to ambient temperature and poured into 200 ml of water. The mixture was acidified with concentrated HCl (pH 5) and extracted with ether (3 × 100 ml). The combined ether extract was dried ($MgSO_4$) and concentrated under reduced pressure leaving a red oil which was crystallized from ether. 1.2 g of **6a** were obtained, mp 133–137°C. Anal. Calcd for $C_9H_8N_2OS$: C: 56.25; H: 4.20; N: 14.58; found: C: 56.32; H: 4.20; N: 14.53.

N,N-*Dimethyl-2-benzoxazolethiocarboxamide* ***(6b)***
Derivative **6b** was prepared by a procedure similar to that described for **6a** starting with **5d** (0.95 g, 5 mmol) and P_2S_5 (1.3 g, 6 mmol). Yield: 7.0 g (70%), mp 72–73.5°C (ether). Anal. Calcd for $C_{10}H_{10}N_2OS$: C: 58.24; H: 4.85; N: 13.58; found: C: 58.21; H: 4.85; N: 13.58.

2-Benzothiazolecarboxanilide ***(8)***
Benzothiazine-2,3-dione [22] (5.64 g, 0.03 mol) was suspended in 75 ml of glacial acetic acid, then aniline (3.62 g, 39 mmol) was added dropwise at room temperature with stirring. The temperature was raised gradually to reflux over a period of 4 h, then the mixture was filtered while still hot. After cooling of the filtrate, the solid precipitate was filtered off yielding 3.7 g (46%) of **8**, mp 153–155°C (acetone) (lit [22] mp 157–158°C).

Rat passive cutaneous anaphylaxis (PCA) assay [23]
Male Sprague-Dawley rate (200–250 g) were injected intradermally (50 µl, i.d.) with 2 dilutions of rat serum containing ovalbumin antibodies at 3 separate sites. After 48 h, the 4 rats in each experimental group were given intraperitoneally 50 mg/kg of the test compound suspended or dissolved in 1 ml of 0.5% methylcellulose (100 centipoise). Control animals received compound vehicle. After 5 min, the rats were challenged intravenously with 4.0 mg of ovalbumin in 1.0 ml of 1.0% Evan's Blue dye in 0.9% saline. Rats were sacrified by cervical dislocation 30 min later, the sites of antibody injection were everted, and the wheal diameters and areas were measured. Mean percent inhibition of wheal size was calculated, and significance of the drug treatment was determined using the Student's *t*-test for unpaired data.

Carrageenin-induced rat paw edema assay
Groups of 6 male Sprague-Dawley rats were given orally test compounds at 30, 40 or 60 mg/kg suspended in 0.5% Methocel. One hour later the right hind paw was injected with 0.1 ml of 1.0% carrageenin. The paw volume was measured at this time and 3 h later. The percent inhibition of edema was calculated.

Dermal vascular permeability assay in rats
Male Sprague-Dawley rats were administered 50 mg/kg of test compound or vehicle by the intraperitoneal (i.p.) route, 5 min before an intraveous (i.v.) injection of 1.0 ml of 1.0% Evan's Blue dye. Ten min later each animal was injected i.d. with 0.1 µg/ml of serotonin in 2 separate sites on the sides of the dorsal midline. On each rat the control sites were injected with physiological saline. Five min after the administration of serotonin the animals were sacrificed and the dorsal skin was cut free to determine the size of the wheal and the effect of the test compound. Each experimental group comprised 4 rats.

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