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Original paper

Synthesis and anti-cholinergic activity of aminoalkyl 2-cyclic amino-2-(1,2-benzisoxazol-3-yl)acetates and their quaternary ammonium salts

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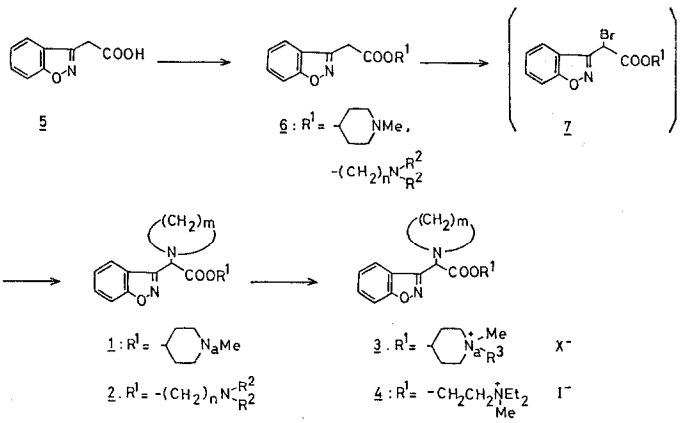
Summary — A series of aminoalkyl 2-cyclic amino-2-(1,2-benzisoxazol-3-yl)acetates **1** and **2** and their quaternary ammonium derivatives **3** and **4** has been prepared and tested for anti-cholinergic activity. Among these compounds, 1-alkyl-1-methyl-4-[2-cyclic amino-2-(1,2-benzisoxazol-3-yl)acetoxy]piperidinium salts **3** showed potent anti-cholinergic activity in the *in vitro* studies. The structure—activity relationships of these compounds are discussed.

Résumé — **Synthèse et activité anti-cholinergique d'une série de cycloamino-2 (benzisoxazol-1,2 yl-3)-2 acétates d'aminoalkyle et leurs sels d'ammonium quaternaires.** *Une série de cycloamino-2 (benzisoxazol-1,2 yl-3)-2 acétates d'aminoalkyle 1 et 2 et leurs dérivés ammoniums quaternaires 3 et 4 a été préparée et essayée pour leur activité anti-cholinergique. Parmi les composés étudiés, le sel d'alkyl-1 methyl-1 [cycloamino-2 (benzisoxazol-1,2 yl-3)-2 acétoxy]-4 piperidinium 3 a fait preuve d'une activité anti-cholinergique puissante* in vitro. *La relation entre la structure et l'activité des composés est discutée.*

1,2-benzisoxazoles / piperidinium salts / stereochemistry / spasmolytic / anti-cholinergic activity

Introduction

Several anti-muscarinic agents are used clinically as spasmolytics. However, there is an unavoidable problem of undesirable anti-cholinergic side effects such as dryness of the mouth and mydriasis. In the search for new and clinically useful anti-muscarinic agents with fewer side effects, we synthesized a series of the title aminoalkyl esters **1** and **2** and their quaternary ammonium salts **3** and **4** as a part of studies on biologically active 1,2-benzisoxazole derivatives [1, 2]. It was found that some of the quaternary ammonium salts **3** and **4**, obtained by highly regioselective quaternization of the corresponding tertiary aminoalkyl esters **1** and **2** showed potent anti-cholinergic activity. This paper deals with the syntheses of these compounds and results of their biological evaluations.



Scheme 1

Chemistry

The title compounds **1**, **2**, **3** and **4** were prepared by the methods shown in Scheme 1. Esterification of 1,2-benzisoxazole-3-acetic acid **5** with appropriate aminoalkanols gave esters **6**. Monobromination of **6** in acetic acid yielded 2-bromoacetates **7**. Amination of **7** with the appropriate cyclic secondary amines gave **1** and **2** shown in Table I. Treatment of *N*-methylpiperidinol derivatives **1** with an excess of the appropriate alkyl halides gave **3** in which only one nitrogen atom (N_a) of the piperidinol moiety

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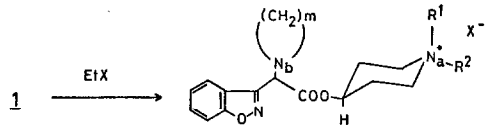
Table I. Aminoalkyl 2-(1,2-benzisoxazol-3-yl)-2-cyclic aminoacetates **1** and **2**.

Compd.	m	R	M.p. °C	(R.Sol.)[a]	Yield[b]	Formula	Anti-ACh[c] ID_{50}(mM)
1a	4		219–222	(A–E)	87	$C_{19}H_{25}N_3O_3 \cdot 2HBr \cdot 0.5H_2O$	3.0×10^{-4}
1b	5		225–230	(AC)	80	$C_{20}H_{27}N_3O_3 \cdot 2HBr$	7.9×10^{-4}
1c	6		207–209	(AC)	87	$C_{21}H_{29}N_3O_3 \cdot HBr$	1.3×10^{-4}
1d	7		170–173	(A–E)	89	$C_{22}H_{31}N_3O_3 \cdot C_2H_2O_4$	2.7×10^{-4}
2a	4	$-CH_2CH_2NEt_2$	170–171	(A–E)	71	$C_{19}H_{27}N_3O_3 \cdot 2HBr$	4.3×10^{-3}
2b	5	$-CH_2CH_2NEt_2$	181–182	(AC)	78	$C_{20}H_{29}N_3O_3 \cdot 2HBr$	5.0×10^{-3}
2c	6	$-CH_2CH_2NEt_2$	134–136	(AC)	77	$C_{21}H_{31}N_3O_3 \cdot 2C_2H_2O_4 \cdot 0.25H_2O$	6.4×10^{-4}
2d	5	$-CH_2CH_2CH_2NMe_2$	151–153	(AC–M)	57	$C_{19}H_{27}N_3O_3 \cdot 2C_2H_2O_4 \cdot 0.75H_2O$	2.4×10^{-3}
2e	6	$-CH_2CH_2CH_2NMe_2$	102–107	(AC)	46	$C_{20}H_{31}N_3O_3 \cdot 2C_2H_2O_4$	9.6×10^{-3}
2f	5	$-CH_2CH_2CH_2NEt_2$	159–160	(AC–M)	73	$C_{22}H_{31}N_3O_3 \cdot C_2H_2O_4$	4.7×10^{-3}
2g	6	$-CH_2CH_2CH_2NEt_2$	144–146	(AC)	65	$C_{22}H_{33}N_3O_3 \cdot C_2H_2O_4$	1.2×10^{-3}
N-Butylscopolamine bromide							2.0×10^{-4}
Atropine sulfate							7.2×10^{-6}

[a] Recrystallization solvent; A = ethanol, E = ether, AC = acetone, IPA = isopropanol.
[b] Yield(%) from **6**.
[c] Concentration required for 50% inhibition of the response by acetylcholine (1.1×10^{-4} mM).

was quaternized as shown in Table II. Treatment of 2-diethylaminoethyl ester **2c** with an equimolar amount of methyl iodide also gave the monoquaternized salt **4**. The site of quaternization of **3** and **4** was deduced from their ^{1}H NMR spectra in which *N*-alkyl proton signals showed significant downfield shifts (*ca.* 1.0—1.2 ppm) in comparison with those of **1** and **2**. The regioselectivity of these quaternizations was mainly attributed to a large difference in the basicity between two nitrogen atoms, N_a and cyclic amino nitrogen atom (N_b). The pK_a values of several aminoalkyl esters were measured by means of a potentiometric titration method [3]. The observed pK_a values were as follows: *e.g.*, compound **1a** (pK_{a1} 8.6 and pK_{a2} 5.0), **1b** (8.5 and 4.4), **2a** (8.8 and 4.7) and **2b** (8.4 and 4.2). Thus, the pK_a value of N_a was about 8.4—8.8, whereas that of N_b was about 4.2—5.0. The unusual decrease in the basicity of N_b may be explained by the presence of the 1,2-benzisoxazole ring whose C=N double bond has been considered to be a masked carbonyl group [4—6].

Quaternization of *N*-methylpiperidinol derivatives **1** with ethyl halides gave an isomeric mixture of *cis*- and *trans*-isomers, **3**-*cis* and **3**-*trans* as shown in Scheme 2. The ratio of these two isomers was determined by ^{1}H NMR spectroscopy in a deuteriochloroform solution of the mixture in which the axial N^+—CH_3 signal of the *trans*-isomer appeared at a higher field than the equatorial N^+—CH_3 signal of the *cis*-isomer [7—11]. The *cis*- and *trans*-isomers showed no difference in the chemical shift of N^+—CH_3 protons in DMSO-d_6. Piperidinium salts



3-*trans*: R^1 = Me, R^2 = Et
3-*cis*: R^1 = Et, R^2 = Me

Scheme 2

3c, **3f**, **3i**, **3j** and **3k** shown in Table II were mixtures of the *cis*- and *trans*-isomers in a ratio about 1: 1 on the basis of integrals of N^+—CH_3 resonance signals of their ^{1}H NMR spectra. Some pure *trans*- and *cis*-isomers were prepared from isomeric mixtures by means of fractional recrystallization.

Pharmacological Results and Discussion

Anti-cholinergic (anti-ACh) activities of the compounds **1**, **2**, **3** and **4** were examined by the inhibitory effect on the response of isolated guinea pig ileum to acetylcholine (ACh), according to the methods previously reported [1, 2]. From the results of anti-ACh activity shown in Tables I and II, some structure—activity relationships of tertiary amino esters **1** and **2** and their quaternary ammonium salts **3** and **4** may be summarized as follows: (1) in regards to the ring size (the number of *m* in **1** and **2**) of the cyclic amino group of **1** and **2**, the order of increasing of potency of these compounds was as follows, $5 < 4 < 7 < 6$ except for two compounds (**2d** and **2e**). (2) Piperidinol derivatives **1** were more active than linear aminoalkanol derivatives **2**. (3) Quaternization of tertiary aminoalkyl esters **1** and **2** giving **3** and **4** produced in an increase of anti-ACh activity.

The anti-ACh activities of the majority of quaternary ammonium salts **3** and **4** were more potent than that of *N*-butylscopolamine bromide. The dose—response curve of ACh in the presence of these compounds was shifted to the right indicating that these compounds antagonized ACh competitively. Among them, some piperidinium salts **3b**, **3d—h**, **3j** and **3l** showed about 1/10 the activity of atropine sulfate and were selected for further pharmacological evaluations.

Other studies have revealed that **3j** has a marked suppressive effect on the spontaneous movement of the stomach in conscious rats and rabbits without systemic side effects of anti-cholinergic origin [12]. Details of the spasmolytic activities of **3j** have been reported elsewhere [13]. Compound **3j** (SX-810) appears to be quite useful as a novel spasmolytic agent which lacks undesirable side effects.

Experimental protocols

Chemistry

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-10 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian

Table II. 1-Alkyl-1-methyl-4-[2-cyclic amino-2-(1,2-benzisoxazol-3-yl)acetoxy]piperidinium salts **3** and **4**.

Compd.	m	R	X	M.p. °C	(R.Sol.)[a]	Yield %	Formula	Anti-ACh[b] ID50(mM)
3a	4		Br	180–182	(A)	52	$C_{20}H_{28}BrN_3O_3$	1.2×10^{-4}
3b	4		I	170–171	(A-E)	71	$C_{20}H_{28}IN_3O_3$	8.0×10^{-5}
3c	4		Br	156–160	(A-E)	61	$C_{21}H_{30}BrN_3O_3$	2.0×10^{-4}
3d	5		I	176–178	(A-M)	80	$C_{21}H_{30}IN_3O_3$	6.4×10^{-5}
3e	5		Br	217–219	(A)	69	$C_{21}H_{30}BrN_3O_3$	6.4×10^{-5}
3f	5		I	180–184	(AC-A-E)	60	$C_{22}H_{32}IN_3O_3$	6.4×10^{-5}
3g	6		I	210–211	(A)	48	$C_{22}H_{32}IN_3O_3$	3.9×10^{-5}
3h	6		Br	204–207	(A-M)	85	$C_{22}H_{32}BrN_3O_3$	4.9×10^{-5}
3i	6		Br	177–178	(AC-E)	92	$C_{23}H_{34}BrN_3O_3$	1.1×10^{-4}
3j	6		I	157–160	(AC-IPE)	90	$C_{23}H_{34}IN_3O_3 \cdot 0.25H_2O$	5.8×10^{-5}
3j-trans	6		I	177–179	(AC)	-	$C_{23}H_{34}IN_3O_3$	3.8×10^{-5}
3j-cis	6		I	173–176	(AC-E)	-	$C_{23}H_{34}IN_3O_3 \cdot 0.5H_2O$	9.6×10^{-5}
3k	6		Tos.[c]	132–140	(AC-EA-E)	70	$C_{23}H_{34}N_3O_3 \cdot C_7H_7O_3S$	-
3l	7		I	195–196	(A-E)	87	$C_{23}H_{34}IN_3O_3$	2.7×10^{-4}
4	6	$-CH_2CH_2N^{+}Et_2$ Me	I	159–161	(AN)	45	$C_{22}H_{34}IN_3O_3$	2.1×10^{-4}

[a] Recrystallization solvent; M = methanol, IPE = isopropylether, AC = acetonitrile, EA = ethyl acetate. See also Table I.
[b] See Table I.
[c] *p*-Toluenesulfonate.

EM-360 or Varian HA-100 spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent and tetramethylsilane as an internal standard. The results of elemental analysis (C, H, Br, Cl, I, N, S) were within ±0.4% of theoretical values.

Aminoalkyl 2-(1,2-benzisoxazol-3-yl)acetate hydrohalide **6**

General procedure. Aminoalkanol (0.035 mol) was added dropwise to a solution of *p*-toluenesulfonyl chloride (0.011 mol) and 1,2-benzisoxazole acetic acid **5** (0.011 mol) in dry toluene (200 ml). The mixture was stirred for 17 h at room temperature and then washed with 5% Na_2CO_3 (200 ml) and with water (200 ml). The toluene was removed *in vacuo* and the residue was dissolved in ether. The ether solution was mixed with conc. hydrohalide. The resulting precipitate was collected by filtration and recrystallized from EtOH—ether to give the title compound **6** (Table III).

Aminoalkyl 2-(1,2-benzisoxazol-3-yl)-2-cyclic aminoacetates **1** *and* **2** *and their salts*

General procedure. A solution of bromine (0.07 mol) in glacial AcOH (20 ml) was added dropwise at 60°C with stirring to a solution of **6** (0.06 mol) in glacial AcOH (80 ml). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in acetone and was diluted with ether. The resulting precipitate was collected and recrystallized from EtOH to give 2-bromoacetate **7**. Cyclic secondary amine (0.05 mol) was added at 10°C with stirring to a solution of **7** (0.01 mol) in $CHCl_3$ (200 ml). After stirring for 20 h at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was basified with saturated Na_2CO_3 solution and was extracted with AcOEt. The solvent was removed *in vacuo* and the residue was converted to hydrobromide or oxalate which was recrystallized from the solvent shown in Table I to give the title compound **1** or **2**.

Table III. Aminoalkyl 2-(1,2-benzisoxazol-3-yl)acetates **6**.

Compd.	R	X	M.p. °C	R.Sol.[a]	Yield[b]	Formula
6a		Br	179–181	A-IPA	80	$C_{15}H_{18}N_2O_3 \cdot HBr \cdot 0.7H_2O$
6b	$-CH_2CH_2NEt_2$	Cl	136–138	A-E	59	$C_{15}H_{20}N_2O_3 \cdot HCl$
6c	$-CH_2CH_2CH_2NMe_2$	Br	111–113	A-E	42	$C_{14}H_{18}N_2O_3 \cdot HBr \cdot 0.25H_2O$
6d	$-CH_2CH_2CH_2NEt_2$	Br	136–138	A-E	51	$C_{16}H_{22}N_2O_3 \cdot HBr$

[a] Recrystallization solvent. See Tables I and II.
[b] Yield(%) from **5**.

1-Alkyl-1-methyl-4-[2-cyclic amino-2-(1,2-benzisoxazol-3-yl)acetoxy]-piperidinium halides **3**

General procedure. Alkyl halide (0.0135 mol) was added to a solution of **1** (free base) (0.003 mol) in acetone (8 ml). The mixture was stirred at room temperature for 5—17 h and concentrated *in vacuo*. The residue was recrystallized from the solvent shown in Table II to give **3**. ^1H NMR ($CDCl_3$) δ ppm: 3.28—3.31 (s, N^+—CH_3 of the *trans*-isomer); 3.43—3.46 (s, N^+—CH_3 of the *cis*-isomer). The ratio of *trans*- to *cis*-isomer was about 1:0.9—1.

2-[2-(1,2-Benzisoxazol-3-yl)-2-(hexahydro-1H-azepin-1-yl)acetoxy]-ethyldiethylmethylammonium iodide ***4***

A solution of **2c** (1.8 g, 0.005 mol) and methyl iodide (0.7 g, 0.005 mol) in acetone (20 ml) was allowed to stand at room temperature for 15 h and then concentrated. The residue was recrystallized from acetone to give **4**, 1.2 g, mp 159—161°C. Anal $C_{22}H_{34}IN_3O_3$ (C, H, I, N).

Preparation of the trans*-isomer of 4-[2-(1,2-benzisoxazol-3-yl)-2-(hexahydro-1H-azepin-1-yl)acetoxy]-1-ethyl-1-methylpiperidinium iodide* ***3j***

A solution of **1c** (6 g, 0.016 mol) and ethyl iodide (12.6 g, 0.08 mol) in acetone (500 ml) was allowed to stand at room temperature for 17 h. The crystalline precipitate was filtrated off. The mother liquor was diluted with ether. Resulting precipitate was collected and recrystallized repeatedly from acetone to give a pure *trans*-isomer of **3j**, 0.29 g, mp 177—179°C. IR (KBr) cm^{-1} 1750. ^1H NMR ($CDCl_3$) δ ppm: 3.28 (3H, s, N^+—CH_3); 5.17 (1H, s, CHN). Anal. $C_{23}H_{34}IN_3O_3$ (C, H, I, N). Attempts to isolate a pure *cis*-isomer from the mother liquor were unsuccessful.

Preparation of the cis*-isomer of 4-[2-(1,2-benzisoxazol-3-yl)-2-(hexahydro-1H-azepin-1-yl) acetoxy]-1-ethyl-1-metylpiperidinium* p*-toluenesulfonate* ***3k***

A solution of **1c** (5 g, 0.0135 mol) and ethyl *p*-toluenesulfonate (13.3 g, 0.0665 mol) in acetone (50 ml) was stirred under reflux for 16 h and then concentrated. From the ^1H NMR spectrum of the crystalline residue (7 g), the composition was estimated to be 50% of the *cis*-isomer of **3k** (N^+—CH_3 peak at 3.30) and 50% of the *trans*-isomer of **3k** (N^+—CH_3 peak at 3.15). Fractional recrystallization of this mixture from acetone—AcOEt—ether gave 45 mg of a pure *cis*-isomer of **3k**, mp 143—144°C. IR (KBr) cm^{-1} 1725. ^1H NMR ($CDCl_3$) δ ppm: 3.30 (3H, s, N^+—CH_3); 5.05 (1H, s, CHN). Anal. $C_{30}H_{41}N_3O_6S$ (C, H, N, S).

Preparation of the cis*-isomer of* ***3j***

A solution of **3k** (0.05 g, 0.9 mmol) and potassium iodide (0.25 g, 1.5 mmol) in a mixture of MeOH (2.5 ml) and water (25 ml) was allowed to stand for 17 h at room temperature and then concentrated. The residue was extracted with $CHCl_3$ and the solvent was removed *in vacuo*. The residue was crystallized from a mixture of acetone and ether to give a *cis*-isomer of **3j**, 0.035 g, mp 173—176°C (dec.). IR (KBr) cm^{-1} 1730. ^1H NMR ($CDCl_3$) δ ppm: 3.45 (3H, s, N^+—CH_3); 5.14 (1H, s, CHN). Anal. $C_{23}H_{34}IN_3O_3 \cdot 0.5H_2O$ (C, H, I, N).

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