Short communication

Indolizine derivatives with biological activity VI
1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine, benanserin structural analogue

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Summary — In continuing the search for new biologically active agents in the indolizine field, 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methyl-indolizine was synthesized and evaluated for its *in vitro* activities on smooth muscle. Anti-histamine, anti-acetylcholine and anti-5-hydroxytryptamine activities, in comparison to those of the indole analogue benanserin, are reported.

Résumé — **Dérivés de l'indolizine à activité biologique VI. 1-(2-Aminoéthyl)-3-benzyl-7-méthoxy-2-méthylindolizine, analogue structural de la benanserine.** Poursuivant les recherches de nouveaux agents biologiquement actifs dans la série des indolizines, l'1-(2-aminoéthyl)-3-benzyl-7-méthoxy-2-méthylindolizine a été synthétisée et testée dans le but d'étudier son activité *in vitro* sur la musculature lisse. Les activités anti-histaminique, anti-acétylcholinique et anti-hydroxy-5 tryptaminique ont été déterminées et comparées avec celles de l'analogue indolique benansérine.

substituted indolizines / 7-methoxy-2-methylindolizines / benanserin analogue / anti-histamine activity / anti-acetylcholine activity / anti-5-hydroxytryptamine activity

Introduction

In previous papers, synthesis and pharmacological evaluation of some aminoethylindolizines, structural analogues of indolylethylamines, are reported [1, 2]. Results obtained made it clear that the indolizine system may be considered as a basis for biologically active compounds, owing to its similarity to indole. Like indole, it is constituted by a delocalized 10 π-electron system to which the aromaticity is attributed. Therefore, it has a theoretical and practical importance. To further explore the structure–activity relationships in this field, 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine **1** has been synthesized. This substance is the exact analogue of the indole derivative benanserin (3-(2-aminoethyl)-1-benzyl-5-methoxy-2-methylindole) **2** (scheme 1) which is a well known active antagonist of 5-hydroxytryptamine (5-HT) [3–5].

The present report deals with direct comparison between *in vitro* anti-histamine, anti-acetylcholine and anti-5-hydroxytryptamine activities of indolizine **1** and indole **2** derivatives.

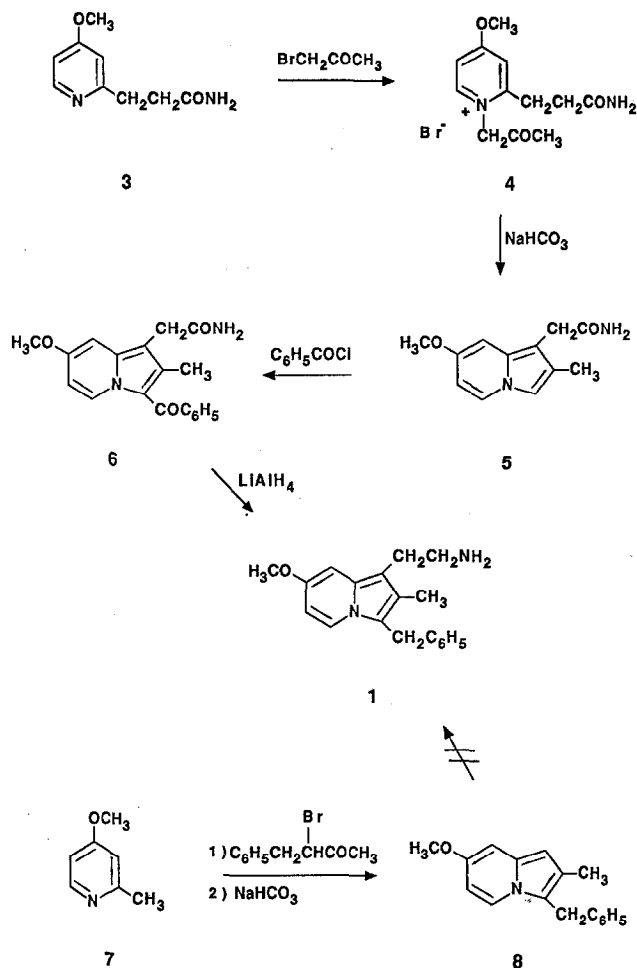*Correspondence and reprints
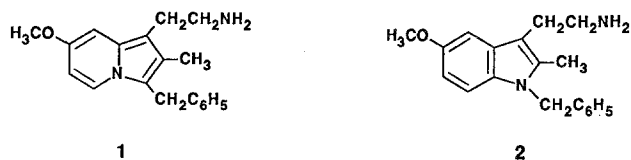
Scheme 1.

Chemistry

The synthesis of the 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine **1** was achieved according to the following procedure (scheme 2).

The reaction of 3-(4-methoxy-2-pyridyl)propionamide **3** with bromoacetone in acetone, followed by cyclization of the pyridinium salt **4** obtained with sodium hydrogen carbonate in ethanol, gave (7-methoxy-2-methylindolizin-1-yl)acetamide **5**. Acylation at the strongly nucleophilic 3 position of the indolizine system was achieved with benzoyl chloride in benzene at room temperature yielding the amide **6**. Reduction of **6** with lithium aluminum hydride in anhydrous ether gave 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine **1**.

Scheme 2.

In an alternative route, the reaction of 4-methoxy-2-picoline **7** [6] with 3-bromo-4-phenyl-2-butanone [7] in absolute ethanol, followed by cyclization of the pyridinium salt obtained with sodium hydrogen carbonate, gave 3-benzyl-7-methoxy-2-methylindolizine **8**. Unfortunately, attempts to introduce groups such as $-CH_2CH_2NO_2$, $-CH_2COOC_2H_5$ and $-COCOCl$ (possible precursors of $-CH_2CH_2NH_2$) into the reactive 1 position of compound **8**, by reaction with nitroethylene, ethyl iodoacetate and oxalyl chloride respectively, failed.

Results and Discussion

The anti-histamine, anti-acetylcholine (using the isolated terminal guinea pig ileum) and anti-5-

hydroxytryptamine (using the isolated rat uterus and rat stomach strips) activities of compounds **1** and **2** were studied *in vitro*.

The 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine **1** inhibited the *in vitro* effects of the 3 agonists histamine, acetylcholine and 5-hydroxytryptamine, employed in our experiments (table I).

The anti-histamine and anti-acetylcholine effects were reliable and easily reproducible, increased with the doses and were completely reversed by washing the organ with the nutrient solution.

The concentration of compound **1** which reduced the effect of 16 ng/ml of histamine by 50% was about 3.6-fold as large as that of benanserin (34.3 *versus* 9.4 µM), the difference being statistically significant ($P < 0.001$).

As far as the anti-acetylcholine effect is concerned, the IC_{50} for compound **1** was 12.8 µM, while that of benanserin was 9.7 µM, that is about 1.3 times lower, the difference being statistically significant ($P < 0.001$).

In the rat stomach, compound **1** produced a dose-dependent inhibition of 5-hydroxytryptamine. The inhibition lasted for a long period and the effect was only in part reversed by repeated washing of the organ with the nutrient solution. Moreover, after the treatment with compound **1**, the sensitivity of the organ to the agonist decreased and never returned to normality, not even after the organ had been repeatedly stimulated with 5-hydroxytryptamine.

In these experiments the IC_{50} for compound **1** was 58.1 µM, while that for benanserin was about 2.6-fold lower, that is 21.7 µM.

The anti-5-hydroxytryptamine effect of compound **1** tested in the rat uterus was not dose-dependent and not even reproducible. In fact, at 162.7 µM, this substance markedly inhibited the effect of 33.3 ng/ml of 5-hydroxytryptamine, but the inhibition was not evoked at lower concentrations and did not increase with larger concentrations of compound **1**. Moreover, after the organ had been exposed to compound **1**, 162.7 µM, neither washing with Tyrode solution nor repeated treatments with the agonist restored the ability of the tissue to respond to 5-hydroxytryptamine. In this respect, the behaviour of compound **1** was exactly the same as that of benanserin described by Shaw and Woolley [4].

Instead, exposure of the rat uterus to concentrations of compound **1** lower than 162.7 µM did not at all affect the sensitivity of the organ to the agonist. For this reason it was not possible to determine the IC_{50} in this test.

The results of present experiments clearly indicate that compound **1** is a non-selective antagonist of histamine, acetylcholine and 5-hydroxytryptamine, and that its activity is always less intense than that of its parent substance, benanserin.

Table I. *In vitro* IC_{50} values[a] determined on different smooth muscle preparations. Figures are means of 6-8 experiments ± SEM.

| | Guinea pig ileum | | Rat stomach |
Compound	Histamine (16 ng/ml)	Acetylcholine (13.8 ng/ml)	5-hydroxytryptamine (2.75 ng/ml)
Atropine		4.5 ± 0.1 nM	
Metergoline			32.9 ± 1.0 nM
Diphenhydramine	22.7 ± 0.7 nM		
Benanserin[b]	9.4 ± 0.3[c] µM	9.7 ± 0.2[c] µM	21.7 ± 1.7[c] µM
1	34.3 ± 0.8[d] µM	12.8 ± 0.4[d] µM	58.1 ± 1.3[d] µM

[a]All concentrations refer to the free base; [b]benanserin was synthesized according to the methods reported by Gaines *et al* [12] and Sletzinger *et al* [13]; [c]difference from comparison standards: $P < 0.001$; [d]difference both from comparison standards and from benanserin: $P < 0.001$

Conclusion

Our data indicate that replacement of the indole with the indolizine system strongly reduces the anti-histamine activity, reduces the anti-5-hydroxytryptamine activity to a lesser extent, and leaves the anti-acetylcholine activity essentially unmodified, the ratios of equieffective dosages of compound **1** to benanserin being 3.6, 2.6 and 1.3 respectively. In any case, the antagonistic activities of both substances appears to be extremely low, being far lower than those of the standards that we employed for comparison.

Compound **1** irreversibly inhibits the effect of 5-hydroxytryptamine in the rat uterus but not in the rat stomach in which, however, it produces a long lasting inhibitory effect. This probably depends on the biological differences existing in the smooth muscles of the 2 organs, but it may also be due to the fact that, as a consequence of its large sensitivity to 5-hydroxytryptamine, the rat stomach was exposed to doses of compound **1** far lower than those employed in the rat uterus.

Experimental protocols

Chemical synthesis

Melting points were taken on a Büchi SPM-120 apparatus and are uncorrected. IR spectra were recorded by a Perkin–Elmer 457 spectrophotometer. ^{1}H NMR spectra were obtained by means of a Varian EM 390 (90 MHz) spectrometer using TMS as the internal standard. All spectral data obtained were considered to be consistent with the assigned structures. The elemental analyses were performed by the Microanalytical Laboratory of the Department of Chemical Sciences of the University of Camerino and were within ± 0.4% of the calculated values.

3-(4-Methoxy-2-pyridyl)propionamide 3

Ethyl 3-(4-methoxy-2-pyridyl)propionate (6.2 mmol) [8] and concentrated aqueous ammonia (5 ml) were well mixed and stored in a stoppered flask in the refrigerator for 1 d. Filtration of the formed crystals, followed by concentration of the filtrate, gave a total of 0.67 g (60% yield) of crystalline amide which, after recrystallization from isopropyl alcohol, melted at 121–123°C.
^{1}H NMR (DMSO–d_6): δ ppm 8.30 (d, 1H, $J = 6.6$ Hz, H-6), 7.20 (bs, 1H, NH), 6.82 (m, 3H, H-3,5, NH), 3.82 (s, 3H, OCH$_3$), 2.92 (m, 2H, CH$_2$), 2.48 (m, 2H, CH$_2$).

1-Acetonyl-2-(2-carbamoyl)ethyl-4-methoxypyridinium bromide 4

A solution of 3-(4-methoxy-2-pyridyl)propionamide **3** (0.01 mol) and 1.8 g (0.013 mol) of freshly distilled bromoacetone was refluxed in acetone (75 ml) for 30 h. The precipitate was filtered and washed with acetone, yielding 2.7 g (80%) of the pyridinium salt. An analytical sample was recrystallized from absolute ethanol, mp: 169–171°C.

(7-Methoxy-2-methylindolizin-1-yl)acetamide 5

A mixture of 2.35 g (7.4 mmol) of the bromide **4** and 2 g of sodium hydrogen carbonate in 100 ml of absolute ethanol was refluxed by stirring under nitrogen for 5 h and filtered. Evaporation of the ethanol gave a solid which was washed with a small amount of water. Recrystallization from *n*-hexane gave 1.42 g (88%) of a white solid, mp: 153–155°C.
^{1}H NMR (CDCl$_3$): δ ppm 7.66 (d, 1H, $J = 7.5$ Hz, H-5), 6.95 (s, 1H, H-3), 6.40 (s, 1H, H-8), 6.17 (dd, 1H, $J = 7.5$ Hz, $J = 2.0$ Hz, H-6), 5.70 (bs, 2H, NH$_2$), 3.78 (s, 3H, OCH$_3$), 3.57 (s, 2H, CH$_2$), 2.20 (s, 3H, 2-CH$_3$).

(3-Benzoyl-7-methoxy-2-methylindolizin-1-yl)acetamide 6

Benzoyl chloride (0.75 ml) was added, while stirring under

nitrogen, to a solution of 1 g (4.58 mmol) of (7-methoxy-2-methylindolizin-1-yl)acetamide **5** in dry benzene (50 ml). Stirring was continued for 3 h at room temperature, then the crude product was collected. Recrystallization from ethanol gave 0.78 g (53%) of a green solid, mp: 253–254°C.

^{1}H NMR (DMSO–d_6): δ ppm 9.49 (dd, 1H, J = 7.5 Hz, J = 2.0 Hz, H-5), 7.47 (s, 5H, Harom), 7.25 and 6.83 (2 bs, 2H, NH$_2$), 7.06 (d, 1H, J = 2.0 Hz, H-8), 6.68 (dd, 1H, J = 7.5 Hz, J = 2.0 Hz, H-6), 3.84 (s, 3H, OCH$_3$), 3.43 (s, 2H, CH$_2$), 1.67 (s, 3H, 2-CH$_3$).

1-(2-Aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine 1

To a suspension of lithium aluminum hydride (10.5 mmol) in anhydrous ether (40 ml) a solution of the amide **6** (2.4 mmol) in dry ether (75 ml) was added slowly. The mixture was refluxed by stirring under nitrogen for 2 h. Aqueous ethanol and 2 N NaOH solution (5 ml) were added. After filtration, the ethereal solution was dried and evaporated. Recrystallization from ethanol gave 0.45 g (65%) of the required compound, mp: 120–121°C. The free base was transformed into the maleate salt by treating a dry ethereal solution of **1** with an equimolar solution of maleic acid in dry ether. This maleate is highly hygroscopic.

^{1}H NMR (DMSO–d_6): δ ppm 7.63 (d, 1H, J = 7.5 Hz, H-5), 7.10 (m, 5H, Harom), 6.64 (d, 1H, J = 2.0 Hz, H-8), 6.01 (dd, 1H, J = 7.5 Hz, J = 2.0 Hz, H-6), 4.16 (s, 2H, ArCH$_2$), 3.70 (s, 3H, OCH$_3$), 2.68 (s, 4H, CH$_2$-CH$_2$), 2.19 (s, 3H, 2-CH$_3$), 1.30 (bs, 2H, NH$_2$).

3-Benzyl-7-methoxy-2-methylindolizine 8

A solution of 4-methoxy-2-picoline (0.08 mol) [6] and 23 g (0.1 mol) of 3-bromo-4-phenyl-2-butanone [7] was refluxed in absolute ethanol (90 ml) for 6 h. The solvent was evaporated and the raw pyridinium salt was dissolved in 100 ml of water. After the addition of sodium hydrogen carbonate (12 g), the mixture was refluxed by stirring under nitrogen for 5 h. Upon cooling, the crude product separated. Recrystallization from ethanol gave 7.15 g (35%) of the required compound, mp: 96–98°C.

^{1}H NMR (CD$_3$COCD$_3$): δ ppm 7.45 (d, 1H, J = 7.5 Hz, H-5), 7.08 (m, 5H, Harom), 6.55 (d, 1H, J = 2.0 Hz, H-8), 6.02 (m, 2H, H-1,6), 4.15 (s, 2H, CH$_2$), 3.65 (s, 3H, OCH$_3$), 2.25 (s, 3H, 2-CH$_3$).

Biological methods

In vitro experiments were carried out according to the following methods:

Guinea pig ileum

A 5 cm segment of the terminal tract of guinea pig ileum was immersed in 15 ml of Krebs solution aerated by air and kept at 32°C, according to Zamboni and Vitali [9].

Rat stomach

The technique described by Vane [10] was employed. A strip of fundus from a rat stomach was suspended in 30 ml of Krebs solution kept at 37°C and aerated by air bubbling through the liquid.

Rat uterus

The technique described by Erspamer [11] was employed. One of the uterine horns of an œstrus rat was suspended in 15 ml of Tyrode solution oxygenated by air and kept at 32°C.

Determination of the antagonistic activity

The dose, which reduced by 50% the response evoked by a standard dose of histamine (16 ng/ml), acetylcholine (13.8 ng/ml) or 5-hydroxytryptamine (2.75 ng/ml, rat stomach, or 33.3 ng/ml, rat uterus) was determined.

The antagonists were introduced into the bath solution, followed 1 min later by the agonists. The time of contact of the antagonists with the organ was 2 (guinea pig ileum), 4 (rat uterus) or 3 min (rat stomach).

Diphenhydramine hydrochloride, atropine sulfate and metergoline maleate were employed as comparison standards. The agonists were histamine dihydrochloride, acetylcholine chloride and 5-hydroxytryptamine creatinine sulfate. Compound **1** and benanserin were employed as maleate and hydrochloride, respectively.

Acknowledgments

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