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Antidiarrhoeal Activity of New Thiazolidinones Related to Loperamide

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Abstract

A series of thiazolidinones related to loperamide was synthesized and evaluated for antidiarrhoeal activity in mice, using the castor oil test.

Of five compounds tested, antidiarrhoeal activity was found only for 2-(*p*-nitrophenyl)-3-{3-[(4-(*p*-chlorophenyl)-4-hydroxy)piperidino]ethyl}-1,3-thiazolidin-4-one. The compound was less active than loperamide (ED50 values = 48·7 (24·8–95·6) and 0·91 (0·24–3·40) mg kg^{-1}, respectively), but was also less toxic (LD50 values = 745·9 (545·2–929·8) and 108·9 (85·5–138·7) mg kg^{-1}, respectively). Its antidiarrhoeal activity was counteracted by naloxone.

Our results support the hypothesis that this compound, like loperamide, is an opiate-receptor agonist.

Loperamide (4-(*p*-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidine butyramide) is at present one of the most efficacious and widely employed antidiarrhoeal drugs; loperamide effectively antagonizes diarrhoea induced by castor oil (Niemegeers et al 1974), prostaglandins (Karim & Adaikan 1977) or cholera toxin (Farack et al 1981). The therapeutic effect of loperamide is believed to be due to its anti-motility and antisecretory properties (Coupar 1987). Opiate antagonists, such as naloxone, prevent some of the intestinal effects of loperamide (Kromer 1988).

Efforts in our laboratories are now directed towards synthetic agents, analogues of loperamide, to gain more knowledge on the structure–activity relationships of the intestine-selective antidiarrhoeal drugs and to develop new antidiarrhoeals. In this regard, we have synthesized five compounds closely related to loperamide, different only by the thiazolidinonic moiety; the length of the methylenic chain between the thiazolidinonic moiety and the piperidine group ranged from two to three groups.

The synthesized compounds were evaluated for their antidiarrhoeal activity using the castor oil test.

Materials and Methods

Synthesis of compounds

The synthetic pathway for the new thiazolidinones is shown in Fig. 1. The thiazolidinones **1–2** and **3–5** were synthesized by condensation of thioglicolic acid and Shiff bases obtained from *p*-substituted benzaldehydes and 4-phenyl-4-hydroxy-1-(3-aminoethyl)piperidine or 4-phenyl-4-hydroxy-l-(3-aminopropyl) piperidine (Diurno et al 1992). Briefly, an equimolar mixture (0·01 mol) of appropriate substituted aldehyde and 4-phenyl-4-hydroxy-l-(3-aminoethyl or aminopropyl) piperidine (0·01 mol) in dry benzene (50 mL) was refluxed until no more water was collected in a Dean–Stark water separator. Mercap-

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Compound	R	n	R′
1	NO_2	2	H
2	NO_2	2	Cl
3	Cl	3	H
4	Cl	3	Cl
5	CH_3	3	Cl

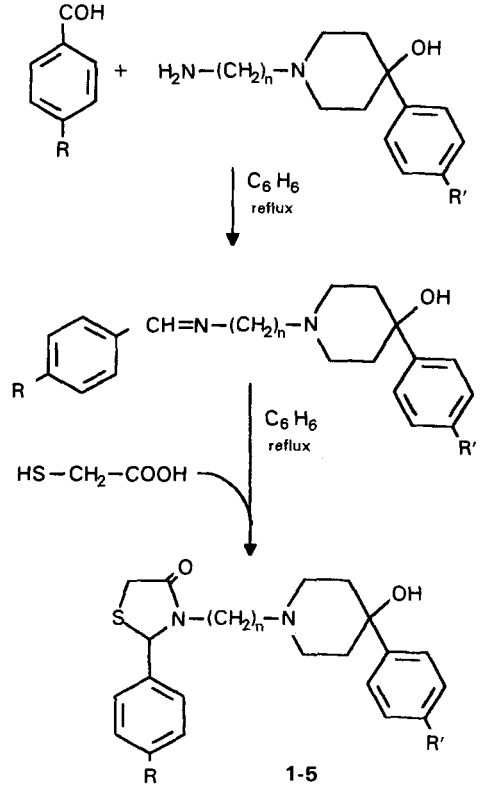

Fig. 1. Synthetic pathway of new thiazolidinones.

toacetic acid (0·01 mol) was added, dropwise, to this crude mixture, and the reaction was carried out at reflux temperature until stoichiometric amounts of water were collected.

The mixtures, cooled and evaporated in-vacuo, afforded, as pale yellow oils, the free bases of **1–5**, which were dissolved in anhydrous ethanol (20 mL). Diethylether (20 mL), saturated with HCl, was added to these solutions. White powders were collected and, recrystallized from dioxane, yielded the thiazolidin-4-one hydrochlorides. Elemental analyses (C, H and N) of the reported compounds were within 0·4% of the theoretical values. NMR and IR spectra were in accordance with their proposed structures. All reactions were routinely checked by thin-layer chromatography.

Castor oil test

Male Swiss mice, 22–25 g (Morini, S. Polo d'Enza, Italy), were housed under standard conditions of lighting with free access to water and food. After a week of acclimatization animals were fasted 12 h before the experiments. Graded doses of compounds synthesized and loperamide (used as reference drug) were given intraperitoneally and 30 min later castor oil (0·2 mL/mouse) was administered intragastrically. Animals were placed in individual cages over clean filter paper. Two hours after oil challenge, mouse cages were inspected (by an observer unaware of the particular treatment) for the presence of the characteristic diarrhoea droppings; their absence was recorded as a protection from diarrhoea (Izzo et al 1994). In some experiments naloxone (Sigma, Milan, Italy) was given subcutaneously (1 mg kg^{-1}) 15 min before the administration of the synthetic compounds. Control mice received vehicle used to dissolve compounds (50% dimethylsulphoxide 0·02 mL/10 g).

Acute toxicity

Male Swiss mice, 22–25 g, were given increasing intraperitoneal doses of the compounds and observed for a time period of 3 days.

Statistics

Antidiarrhoeal activity was evaluated using the chi-square test; a $P < 0·05$ was considered significant. LD50 and ED50 (95% confidence limits) were determined by probit analysis.

Results

Compounds **3**, **4** and **5** showed very high toxicity (LD50 values in mg kg^{-1}: compound **3**, 12·5 (9·2–11·2); compound **4**, 7·7 (5·4–11·2); compound **5**, 5·7 (4·5–7·4), n = 7–8) while compounds **1** and **2** showed lower toxicity compound **1**, 691·2 (486·8–1141); compound **2**, 745·9 (545·2–929·8)) compared with loperamide (108·9 (85·5–138·7)).

However compounds **3**, **4** and **5** were inactive at doses of 0·5–2·5 mg kg^{-1} and compound **1** was inactive at doses of 10–250 mg kg^{-1}, whereas compound **2** reduced castor oil-induced diarrhoea in a dose-dependent manner: ED50 in mg kg^{-1} 48·7 (24·8–95·6)); the lower doses (1–25 mg kg^{-1}) produced a weak effect, while the higher doses (50 and 100 mg kg^{-1}) significantly inhibited diarrhoea (50–80% protection).

Naloxone (1 mg kg^{-1}, s.c.) given alone was without effect, but significantly reversed the antidiarrhoeal activity of 100 mg kg^{-1} compound **2** (Table 1). However, 5 mg kg^{-1} loperamide inhibited castor oil-induced diarrhoea by 90% (ED50 = 0·91 (0·24–3·40) mg kg^{-1}).

Table 1. Effect of intraperitoneal doses of compound **2** (alone or together with naloxone 1 mg kg^{-1}, s.c.) and loperamide on castor oil-induced diarrhoea 2 h after its oral administration (0·2 mL/mouse).

Treatment	Dose (mg kg^{-1})	Number of mice with diarrhoea
Control		0/10
Naloxone	1	0/10
Loperamide	5	0/10
Castor oil		10/10
Castor oil + compound **2**	10	9/10
	25	8/10
	50	5/10*
	100	2/10**
Castor oil + compound **2**	100	
+ naloxone	1	8/10#
+ loperamide	5	1/10**

*$P < 0·05$, $P < 0·01$ compared with castor oil, #$P < 0·05$ compared with castor oil + 100 mg kg^{-1} compound **2**.

Discussion

The castor oil test has been used extensively in our laboratories as a basic pharmacological test to study the role of endogenous substances involved in diarrhoea and to screen antidiarrhoeal drugs. One of the assets of this model is the very reproducible evacuation of watery stools 2-h after castor oil administration. In addition we have studied a 2-h time-period after castor oil because at this time all mice exhibited copious diarrhoea.

Of five compounds studied, only one (compound **2**) possessed antidiarrhoeal activity with an ED50 value about one-fifteenth that of the acute LD50. This compound, compared with loperamide, was one-fiftieth as active as the reference compound, but about one-seventh as toxic.

Moreover, the *p*-chlorine substituent of phenyl group on the 4-position of the piperidine ring seems to be essential for the biological activity (compare compounds **1** and **2**). Naloxone, considered to be a relatively pure opiate-receptor antagonist, antagonizes the antidiarrhoeal action of compound **2** probably owing to the opiate-receptor-mediated blockade. Such a conclusion is consistent with the observations that loperamide competes with naloxone for binding to opiate receptors of both brain and gut (Wüster & Hurz 1978; Kromer 1988).

On this basis, models of loperamide and compound **2** were constructed using PC Model, minimized by an MMX force-field calculation (PC Model Serena software, Bloomington, IN) and superimposed. The alkylamine chain of compound **2** fits with the chain of loperamide, while the overlap of carbonyl and phenyl of the thiazolidinonic moiety with the corresponding groups of the loperamide shows only 0·489 Å, for the average deviation of atoms.

The lower activity of compounds **3–5** with a propyl group linking the piperidine and thiazolidinone rings could depend on

the length of the chain; in fact the superimposition of compound **3** with loperamide showed a lack of fitting of the molecules.

These results support the hypothesis that compound **2**, like loperamide, is an opiate-receptor agonist.

The very close Rf values of compound **2** (0·44) and loperamide (0·48) obtained by TLC analysis on silica gel using *n*-exan-triethylamine-ethanol (10 : 2 : 3) indicate that both molecules have similar lipophilicity and could have similar pharmacokinetics.

In this regard studies are in progress to synthesize new molecules with different substituents.

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