Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 4, pp. 477-480, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00052-2

(R)-3-(6-CHLORO-1-ISOPROPYLBENZIMIDAZOLE-4-CARBOXAMIDO)QUINUCLIDINE: A HIGH AFFINITY LIGAND FOR THE (R)-ZACOPRIDE BINDING SITE

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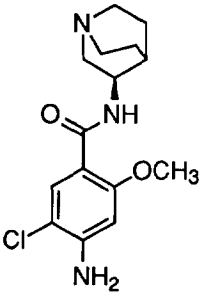
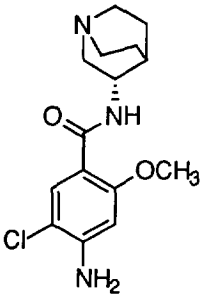
Abstract: The (R)-3-amido quinuclidine **6** (RS-16566) was found to be a high affinity ligand for the (R)-zacopride binding site.

Zacopride is a well-known racemic serotonergic agent that exhibits properties of both $5HT_3$ antagonism[3] and $5HT_4$ agonism.[4] The order of binding affinity of the zacopride antipodes is (S)-zacopride > (R)-zacopride at both of these serotonin receptor subtypes; however,recent reports have described a non-serotonergic binding site in rat cerebral cortex and NG 108-15 clonal cells that exhibits (R)-zacopride > (S)-zacopride selectivity.[5,6] The extant pharmacology and distribution studies of the so-called (R)-zacopride binding site suggest a novel, high affinity locus with a wide distribution in central and peripheral tissues.[5b] This binding site has so far been primarily characterized by radioligand binding studies with [^{3}H]-(R)-zacopride; however, emerging in vivo data suggest that it may exhibit a functional role in the behavioral actions of (R)-zacopride that is not simply accounted for by serotonergic properties.[7]

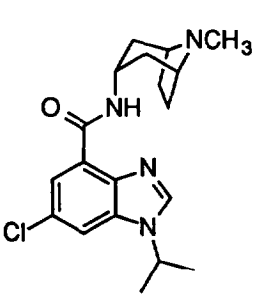


(R)-zacopride

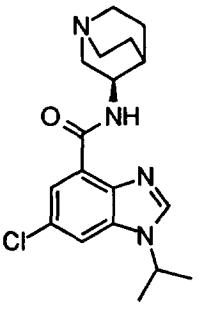


(S)-zacopride

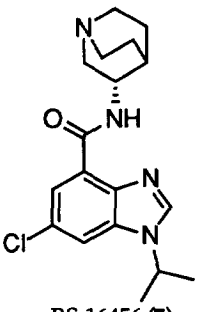
During the course of a program aimed at discovery of $5HT_3$ and $5HT_4$ receptor ligands we noted that the achiral lead RS-33800, endo-3-(6-chloro-1-isopropylbenzimidazole-4-carboxamido)tropane (**5**), displayed a relatively high affinity (16 nM) for the (R)-zacopride binding site ((R)-ZBS) in NG 108-15 clonal cells. The binding affinity of **5** is roughly comparable to that of (R)-zacopride itself using the NG 108-15 cell line; therefore, we briefly explored the effect of substituting (R)-3-quinuclidinyl and (S)-3-quinuclidinyl moieties for the tropane ring system to give the enantiomeric amides RS-16566 (**6**) and RS-16456 (**7**), respectively.



RS-33800 (5)



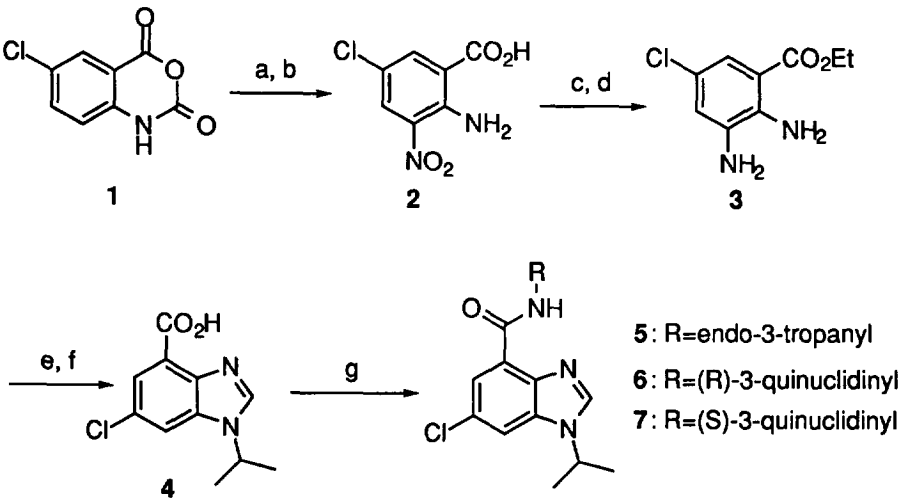
RS-16566 (6)



RS-16456 (7)

The 6-chloro-1-isopropylbenzimidazole-4-carboxamides **5-7** were prepared from commercially available 5-chloroisatoic anhydride (**1**) as shown in Scheme I. Nitration of **1**, followed by hydrolysis of the anhydride group, gave 5-chloro-3-nitroanthranilic acid **2** in 80 % yield. Esterification of compound **2** with satd. ethanolic HCl followed by reduction of the nitro group with H_2-10 % Pd/C afforded ethyl 5-chloro-3-aminoanthranilate **3** in quantitative yield. The 3-amino group was selectively monoalkylated by warming a solution of **3** in 1:1 2-iodopropane-DMF to 50 °C for 6 h; chromatographic purification of the N-monoalkylated product, followed by treatment with formic acid in hot aq. HCl, gave 6-chloro-1-isopropylbenzimidazole-4-carboxylic acid (**4**) in 35 % yield. Carboxylic acid **4** was coupled with endo-3-aminotropane, (R)-3-aminoquinuclidine, or (S)-3-aminoquinuclidine using carbonyl diimidazole in DMF solution to afford amides **5-7**, respectively. Compounds **5-7** were converted to their hydrochloride salt forms for all subsequent uses.

Scheme I



a: $NaNO_3/H_2SO_4$ **b**: H_2O, reflux **c**: EtOH-HCl **d**: H_2-10 % Pd/C **e**: 1:1 DMF-2-iodopropane (50 °C, 6 h) **f**: HCO_2H-aq. HCl, reflux **g**: CDI-DMF; then RNH_2

Selected receptor binding affinities for compounds **5**-**7** and the zacopride enantiomers are given in the Table.

TABLE. Receptor Binding Affinities of **5**-**7** and (R)- and (S)-zacopride

	Binding pK_i[a]		
Compound	$5HT_3$[b]	$5HT_4$[c]	(R)-ZBS[d]
5	9.0±0.1	6.80±0.07	7.70±0.10
6	9.27±0.03	7.62±0.01	9.84±0.42
7	9.86±0.08	6.8[e]	6.22±0.30
(S)-zacopride	9.74±0.03	6.36±0.12	5.3[f]
(R)-zacopride	8.43[g]	5.55±0.13	8.30±0.22

[a]Except as noted, radioligand binding assays were carried out as described in reference 8. Values are mean pK_i±SEM ($n \geq 3$).
[b]Displacement of [^{3}H]-BRL 43694 from NG-108-15 cell membranes.
[c]Displacement of [^{3}H]-GR 113808 from Guinea pig brain striatum.
[d]Displacement of [^{3}H]-(R)-zacopride from ondansetron-treated NG-108-15 cell membranes. [e]pK_b estimate from relaxation of carbachol-contracted rat esophageal muscularis mucosae. [f]Reference 5.
[g]Displacement of racemic [^{3}H]-zacopride from rat cortex (reference 3b).

All of the compounds in the Table exhibit high affinity for the $5HT_3$ receptor and low-to-moderate affinity for the $5HT_4$ receptor; however,the range of affinities of these compounds at the (R)-zacopride binding site ((R)-ZBS) is remarkable. Thus, the (R)-antipode of zacopride shows a 1000-fold higher (R)-ZBS affinity than does (S)-zacopride.[5] (R)-Amido quinuclidine **6**, which shows 4000-fold higher affinity at the (R)-zacopride binding site than the corresponding (S)-antipode **7**, also exhibits a 35-fold improvement in (R)-ZBS binding affinity over (R)-zacopride itself. Unfortunately, while both (S)-zacopride and compound **7** show excellent $5HT_3$/(R)-ZBS selectivity,no ligands are known to date which exhibit useful (R)-ZBS/$5HT_3$ selectivity. The achievement of *ca.* 100-fold (R)-ZBS/$5HT_3$ selectivity in a high affinity ligand would clearly provide a powerful pharmacological tool for the further assessment of physiological functions of the (R)-zacopride binding site; thus, additional structure-activity studies are needed toward this end.

References and Notes

[Ψ]This paper is dedicated to Professor Clayton H. Heathcock on the occasion of his 60th birthday.

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8. **$5HT_3$ receptor binding assay:** NG-108 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10 % bovine calf serum and 1 X hypoxanthine-aminopterin-thymidine. Confluent cells were harvested from 250 mL flasks using a 1 min exposure to trypsin. The cell suspension was centrifuged (200 x G; 5 min), resuspended in a Tris (50 nM) EDTA (5 mM) buffer (pH 7.4 @ 4 °C), homogenized using a Polytron P10 tissue disrupter (setting 5 for 10 sec), and recentrifuged for 15 min. For competition binding assays the NG-108 cell membranes were incubated with 1.0 nM [^{3}H]-BRL 43694 and competing ligands in 0.25 mL of Tris-Krebs buffer (154 mM NaCl, 5.4 mM Kcl, 1.2 mM K_2PO_4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM D-glucose and 10 mM Tris; pH 7.4 @ 25 °C) for 45 min. Reactions were terminated by vacuum filtration through filters pretreated with 0.3 % PEI. Nonspecific binding was determined with 1 μM BRL 43694. **(R)-zacopride binding site assay:** NG-108 cell membranes, prepared as above, were incubated with 2 nM [^{3}H]-(R)-zacopride and competing ligands in 0.5 mL of Tris buffer (25 mM Tris-HCl; pH 7.4 @ 30 °C). 1 μM Ondansetron was included in the incubation to prevent [^{3}H]-(R)-zacopride binding to $5HT_3$ receptors. Reactions were terminated after 30 min by vacuum filtration. Nonspecific binding was defined with 100 μ mianserin. **$5HT_4$ receptor binding assay:** Guinea pig brain striata were dissected from Guinea pig brains (Rockland Inc., Gilbertsville, PA). Membranes were prepared by homogenization in a Tris buffer (10 mM Tris, 250 mM sucrose, 5 mM EDTA; pH 7.4 @ 4 °C), filtration through nylon mesh, and centrifugation of the filtrate (1000 x G for 15 min). Membranes were incubated with 0.1 nM [^{3}H]-GR 113808 and competing ligands in 0.5 mL of a HEPES buffer (50 mM HEPES, 130 mM choline chloride, 5 mM D-glucose, 5.4 mM Kcl, 0.5 mM EDTA; pH 7.4 @ 25 °C). Reactions were terminated after 1 h by vacuum filtration. Nonspecific binding was defined with 1 μM GR 113808.

(*Received in USA* 23 *December* 1995; *accepted* 23 *January* 1996)