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Synthesis and Pharmacology of Isoquinuclidine Derivatives as 5-HT_3 Ligands

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Abstract—A series of 4-amino-5-chloro-2-methoxybenzoates and benzamides containing the 5- and 6-isoquinuclidinyl system was synthesised and evaluated for binding to 5-HT_3, 5-HT_4 and D_2 receptors. In general, the isoquinuclidine derivatives at the 5-position have shown to be more potent as 5-HT_3 ligands but they also possess 5-HT_4 and D_2 properties. However, the results show that the derivatives at the 6-position afforded the most promising compounds in terms of both receptor affinity and selectivity.

A number of potent 5-HT_3 antagonists have been reported and shown to be effective in the control of cancer chemotherapy-induced emesis.[1] It was clear from the earliest discovery that there were probably three pharmacophore elements required for high affinity: an aromatic ring at an appropriate distance from a carbonyl or bioisoster connected to a basic centre. It can be assumed that the basic centre binds in its protonated form. Different QSAR and modelling studies have been made to delineate this pharmacophore and to determine angles and distances.[2]

There are many variations referring to the aromatic component, the linkers and the basic side chain. Our own interest came from the observation that metoclopramide (Fig. 1) had three major identified pharmacological activities: dopamine antagonism, 5-HT_3 receptor antagonism, and gastric motility stimulant properties (5-HT_4 agonism).[1] We decided to synthesise a series of amides to investigate the effect that the replacement of the diethylaminoethyl chain by a 2-azabicyclo[2.2.2]-octane (isoquinuclidine) system could exert in their pharmacological properties.

Furthermore, the isoquinuclidine system has not been much used as basic side chain in such a class of ligands. To our knowledge, only five compounds with this system have been reported as 5-HT_3 antagonists and no systematical synthesis is described.[3] We also synthesised the equivalent esters **5**, **6** and **11**, **12** due to the fact that it can be possible to convert the agonist/partial agonist 5-HT_4 properties of the benzamides into antagonists when the amidic function is replaced by an ester function. The first published indication that this can be done came from the finding that the ester equivalent of metoclopramide, SDZ 205-557, had antagonist activity in a number of pharmacological models.[1]

In this paper, we compare the esters and the amides in terms of both receptor affinity and selectivity for the D_2, 5-HT_3 and 5-HT_4 receptors.

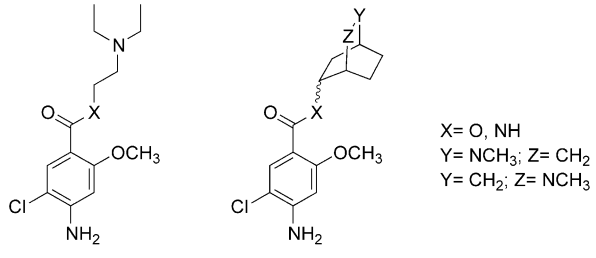

Figure 1. Metoclopramide, SDZ 205-557 and the general structure for compounds **5**, **6**, **11**, **12** and **17**–**20**.

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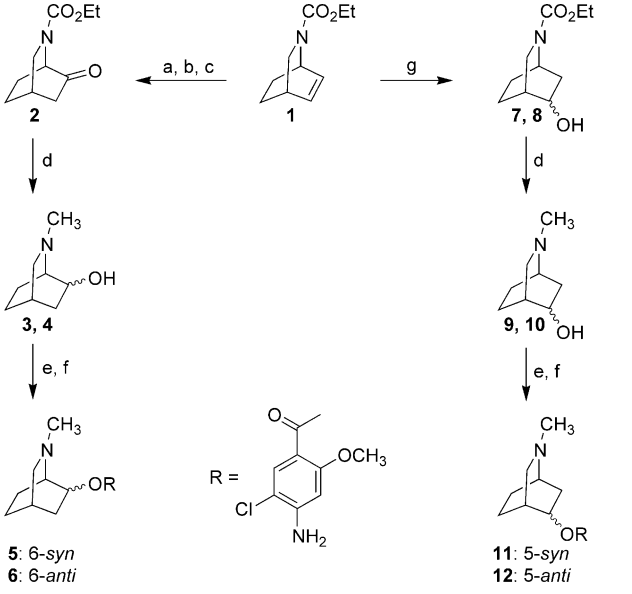
Chemistry

The key intermediates in the synthesis of esters **5**, **6** and **11**, **12** were, of course, the corresponding alcohols **3**, **4**, **9** and **10**. Two basic approaches were utilised and are shown in Scheme 1. The synthesis of alcohols **3** and **4** was accomplished by reduction with lithium aluminium hydride of 2-carbethoxy-2-azabicyclo[2.2.2]oct-6-one (**2**) which was prepared by the method of Krow et al.[4] This method involves the addition of phenylselenenyl chloride to 2-carbethoxy-2-azabicyclo[2.2.2]oct-5-ene (**1**) followed by dehydrohalogenation and hydrolysis of the derived vinyl selenide to give regioselectively the above mentioned compound. Reduction of this compound gave two different alcohols **3** and **4** in a ratio of 40/60 based on NMR data. The alcohols **9** and **10** were obtained by the procedure described by Krow,[5] using again the 2-carbethoxy-2-azabicyclo[2.2.2]oct-5-ene (**1**) but in this case an oxymercuration-demercuration procedure was applied. The addition of mercuric acetate to the alkene, followed by the reduction of the mercuric intermediate with $NaBH_4$, gave an epimeric mixture of 5-*syn*- and 5-*anti*-2-ethoxycarbonyl-2-azabicyclo[2.2.2]-octanols (**7**, **8**) in a ratio of 50/50 based on NMR data. Finally, the alcohols **9** and **10** can be obtained by reduction of this epimeric mixture with $LiAlH_4$.

The tritylated benzoate derivatives were synthesised in a straightforward manner by esterifying the corresponding mixture of alcohols in the presence of DBU with 5-chloro-4-(tritylamino)-2-methoxybenzoic acid imidazolide previously synthesised.[6,7] The resulting residue was chromatographed on silica gel with the appropriate solvent system to separate each of the epimers of the corresponding esters. This procedure gave slightly better yields than the one used by Fernandez et al.[8] where ^{n}BuLi as base was used instead of DBU. Treatment of those esters with HCl produced the trityl group cleavage and the compounds could be obtained as hydrochloride salts. To synthesise the free base, treatment with a Na_2CO_3 solution was necessary.



Scheme 1. (a) ClSePh, CH_2Cl_2, −78 °C and then 15 h at rt; (b) DBU, 140 °C for 15 h; (c) HCl (20%), dioxan, 65 °C for 48 h; (d) $LiAlH_4$, THF, reflux for 24 h; (e) 5-chloro-2-methoxy-4-(tritylamino)benzoic acid imidazolide, DBU, THF, reflux for 24 h; (f) HCl, $CHCl_3$, rt, 12 h; (g) $Hg(Aco)_2$, THF–H_2O (1:1), rt for 24 h, then 3 M NaOH, $NaBH_4$ (in 3 M NaOH).

Once alcohols have been synthesised the easiest procedure to obtain the amines is by reduction of the azides which can be obtained from the alcohols through mesilates. In the case of compounds **15** and **16**, alcohols **7** and **8** were used as starting materials since the reduction of the azide and the carbethoxy group can be accomplished at the same time, this approach would avoid one reaction step (Scheme 2).

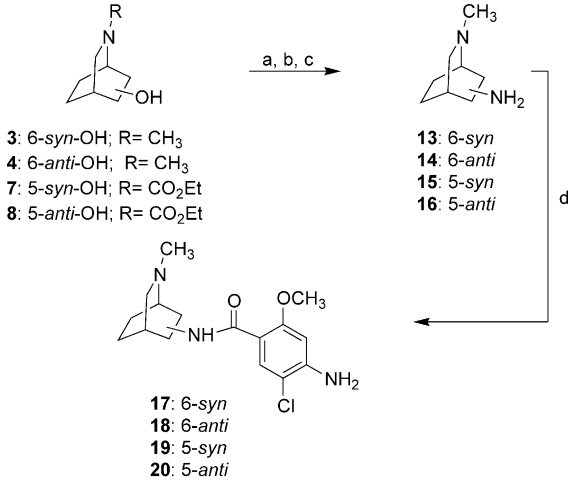
Finally, by reaction of the corresponding amines with the 4-amino-5-chloro-2-methoxybenzoic acid using ethyl chloroformate in presence of triethylamine, the amides **17**–**20** were obtained.[9] The epimeric mixtures were separated by column chromatography on silica-gel.

Pharmacology

Even though all the compounds are racemic a preliminary pharmacological evaluation was carried out on compounds **5**, **6**, **11**, **12** and **17**–**20** using granisetron, lerisetron and tropisetron as reference compounds (Table 1).[10] Compounds **6**, **11**, **12** and **20** were found to show high affinity for the 5-HT_3 receptor. The esters derivatives **11** and **12** in spite of being the most potent 5-HT_3 ligands are not selective because they showed also 5-HT_4 properties.

The amides **19** and **20** are dopaminergic ligands, as it had been published by Blaney et al.,[11] showing the latter compound 5-HT_3 properties.

Finally, compound **6** can be identified as a moderately potent and selective 5-HT_3 receptor ligand.



Scheme 2. (a) Et_3N, CH_2Cl_2, 0 °C, then $ClSO_2CH_3$, rt for 1.5 h; (b) NaN_3, *N*-methyl-2-pirrolidone, 150 °C for 1 h; (c) $LiAlH_4$, THF, reflux for 5 h; (d) 4-amino-5-chloro-2-methoxybenzoic acid, ethyl chloroformate, Et_3N, CH_2Cl_2, rt for 12 h.

Table 1. Binding affinities at the 5-HT_3, 5-HT_4 and D_2 receptors for compounds **5**–**6**, **11**–**12** and **17**–**20**

Compd	Binding 5-HT_3 IC_{50} (μM)[a]	Binding 5-HT_4 IC_{50} (μM)[a]	Binding D_2 IC_{50} (μM)[a]
5	191.8 (±27.3)[b]	>1	≫1
6	13.79 (±1.81)[b]	≫1	≫1
11	11.17 (±0.85)[b]	240.3 (±30.6)[b]	≫1
12	5.19 (±0.65)[b]	82.55 (±7.92)[b]	≫1
17	≫1	≫1	1
18	327.2 (±32.8)[b]	1	≫1
19	1	1	842.2 (±47.1)[b]
20	17.26 (±2.25)[b]	≫1	130.3 (±10.8)[b]
Granisetron	1.76 (±0.15)[b]	>1	>1
Lerisetron	0.62 (±0.04)[b]	>1	>1
Tropisetron	1.55 (±0.10)[b]	125.1[b]	>1

[a]Values are means of three experiments, standard deviation is given in parentheses.
[b]K_i value (nM).

As far as 5-HT_3 affinity is concerned, the following conclusions can be drawn:

a. Compounds containing the ester linkage (**5**, **6**, **11** and **12**) are more potent than those containing the amide one (**17**–**20**).
b. *Anti* steroisomers (amides and esters) were found to be more potent than *syn* steroisomers.

In summary, the interesting biological profile of compound **6**, suggests that it would be promising to study further the individual enantiomers of these systems and also to determine the agonistic or antagonistic properties.

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7. General procedure for the preparation of 4-amino-5-chloro-2-methoxybenzoates derivatives **5**, **6**, **11** and **12**: To a solution of recently prepared 5-chloro-2-methoxy-4-tritylaminobenzoic acid imidazolide (1 equiv) in dry THF was added a solution of the alcohols (epimeric mixture) (1 equiv) in DBU (1 equiv). The mixture was refluxed for 24 h and concentrated under pressure to dryness. The residue was taken into CH_2Cl_2, washed with water and dried over $MgSO_4$ to obtain a mixture of the corresponding *syn* and *anti* 5-chloro-2-methoxy-4-tritylaminobenzoate derivatives, which were separated by column chromatography on silica gel. For the 6-isoquinuclidinyl derivatives a mixture of $CHCl_3/CH_3OH$ (95/5) was used as eluent to obtain the 2-methyl-2-azabicyclo[2.2.2]octan-6-*syn*-yl 5-chloro-2-methoxy-4-tritylaminobenzoate (37%) as the first-eluting epimer and the 2-methyl-2-azabicyclo[2.2.2]octan-6-*anti*-yl 5-chloro-2-methoxy-4-tritylaminobenzoate (35%) as the second one. For the 5-isoquinuclidinyl derivatives a mixture of $CHCl_3/CH_3OH$ (95/5) was employed to obtain the 2-methyl-2-azabicyclo[2.2.2]octan-5-*anti*-yl 5-chloro-2-methoxy-4-tritylaminobenzoate (43%) as the first-eluting epimer. Continued elution gave the 2-methyl-2-azabicyclo[2.2.2]octan-5-*syn*-yl 5-chloro-2-methoxy-4-tritylaminobenzoate (16%).

Concentrated HCl (2 equiv) was added to a solution of the tritylated ester (1 equiv) in $CHCl_3$. The reaction was stirred for 12 h and then concentrated under reduced pressure to dryness. The mixture was taken up with water, basified with Na_2CO_3 and then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent removed to afford a solid which was purified by column chromatography on silica gel to obtain the corresponding non-tritylated benzoate. In compounds **5** and **6**, $CHCl_3/CH_3OH$ (9/1) was employed as eluent while $CHCl_3/CH_3OH$ (95/5) was used for compounds **11** and **12**. **5**: Yield: 61%; mp 126 °C (decomp); ^1H NMR ($CDCl_3$) δ 7.85 (s, 1H, Har), 6.27 (s, 1H, Har), 4.93 (m, 1H, H-6), 4.44 (brs, 2H, NH_2), 3.83 (s, 3H, OCH_3), 2.99 (m, 1H, H-3n), 2.86 (m, 1H, H-1), 2.62 (m, 1H, H-3x), 2.49 (s, 3H, NCH_3), 2.17 (m, 1H, H-5a), 2.04 (m, 1H, H-7s), 1.83 (m, 1H, H-4), 1.74 (m, 1H, H-5s), 1.62 (m, 1H, H-8s), 1.51 (m, 1H, H-7a), 1.44 (m, 1H, H8a). Anal. calcd for $C_{16}H_{21}ClN_2O_3$: C, 59.16; H, 6.52; N, 8.63; found: C, 59.22; H, 6.58; N, 8.64. **6**: Yield: 70%; mp 72–75 °C; ^1H NMR ($CDCl_3$) δ 7.78 (s, 1H, Har), 6.28 (s, 1H, Har), 5.24 (m, 1H, H-6s), 4.46 (brs, 2H, NH_2), 3.83 (s, 3H, OCH_3), 2.83 (m, 1H, H-1), 2.73 (m, 1H, H-3n), 2.66 (m, 1H, H-3x), 2.47 (s, 3H, NCH_3), 2.23 (m, 1H, H-5s), 1.86 (m, 2H, H-7a, H-7s), 1.80 (m, 1H, H-4), 1.62 (m, 2H, H-8a, H-8s), 1.57 (m, 1H, H-5a). Anal. found: C, 59.12; H, 6.44; N, 8.73. **11**: Yield; 42%; mp 138–140 °C; ^1H NMR ($CDCl_3$) δ 7.82 (s, 1H, Har), 6.26 (s, 1H, Har), 5.00 (m, 1H, H-5), 4.41 (brs, 2H, NH_2), 3.83 (s, 3H, OCH_3), 3.03 (m, 1H, H-3n), 2.70 (m, 1H, H-3x), 2.57 (m, 1H, H-1), 2.39 (s, 3H, NCH_3), 2.02 (m, 3H, H-6a, H-6s, H-7s), 1.91 (m, 1H, H-4), 1.74 (m, 1H, H-8s), 1.64 (m, 1H, H-8a), 1.40 (m, 1H, H-7a). Anal. found: C, 59.33; H, 6.71; N, 8.69. **12**: Yield: 32%; mp 148–149 °C; ^1H NMR ($CDCl_3$) δ 7.79 (s, 1H, Har), 6.29 (s, 1H, Har), 5.14 (m, 1H, H-5), 4.78 (brs, 2H, NH_2), 3.85 (s, 3H, OCH_3), 3.00 (m, 1H, H-3x), 2.71 (m, 1H, H-1), 2.66 (m, 1H, H-3n), 2.56 (m, 1H, H-6s), 2.26 (s, 3H, NCH_3), 2.10 (m, 1H, H-7s), 2.08 (m, 1H, H-4), 1.97 (m, 1H, H-8a), 1.65 (m, 2H, H-7a, H-8s), 1.54 (m, 1H, H-6a). Anal. found: C, 59.29; H, 6.38; N, 8.51.
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9. General procedure for the preparation of 4-amino-5-chloro-2-methoxybenzamide derivatives **17**–**20**: An ethyl chloroformate (1 equiv) solution in anhydrous CH_2Cl_2 was slowly added (15 min) over a mixture of Et_3N (1 equiv) and 4-amino-5-chloro-2-methoxybenzoic acid (1 equiv) in dry CH_2Cl_2. The mixture was stirred at room temperature for 30 min and then a solution of the corresponding amines (1 equiv) in dry CH_2Cl_2 was added. The stirring was continued during 12 h and the mixture was basified with 2.5 N NaOH. After 30 min the organic layer was separated, washed with brine and dried over Na_2CO_3. The clear solution was concentrated under reduced pressure to give the corresponding amides mixture which were separated by silica gel chromatography using the adequate solvents. For the 6-isoquinuclidinyl derivatives a mixture of $CHCl_3/CH_3OH$ (ammonia satured) (97/3) was used to obtain the first-eluting compound **17**: Yield:

7%; mp 163–166 °C (decomp); ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1H, Har), 7.98 (m, 1H, NH amide), 6.30 (s, 1H, Har), 4.35 (brs, 2H, NH_2), 3.88 (s, 3H, OCH_3), 3.75 (m, 1H, H-6a), 3.24 (m, 1H, H5s), 2.70 (m, 1H, H-3n), 2.28 (s, 3H, NCH_3), 2.22 (m, 1H, H-3x), 2.12 (m, 2H, H-1, H-5a), 1.85 (m, 1H, H-7s), 1.70–1.40 (m, 4H, H-4, H-7a, H-8a, H-8s). Anal. calcd for $C_{16}H_{22}ClN_3O_2$: C, 59.34; H, 6.85; N, 12.98; found: C, 59.17; H, 6.63; N, 12.71. Continued elution gave compound **18**: Yield: 29%; mp 94–96 °C (decomp); ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H, Har), 7.65 (m, 1H, NH amide) 6.27 (s, 1H, Har), 4.38 (brs, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.68 (m, 1H, H-6s), 3.36 (m, 1H, H5a), 2.74 (m, 1H, H-1), 2.52 (m, 1H, H-3n), 2.37 (s, 3H, NCH_3), 2.22–2.04 (m, 2H, H-5s, H-7a), 1.98 (m, 1H, H-3x), 1.84–1.42 (m, 4H, H-4, H-7s, H-8a, H-8s). Anal. found: C, 59.22; H, 6.71; N, 12.80. For the 5-isoquinuclidinyl derivatives a mixture of $CHCl_3$/CH_3OH (ammonia satured) (98/2) was used to obtain the first-eluting compound **20**: Yield: 27%; mp 158–161 °C; ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H, Har), 7.85 (m, 1H, NH amide), 6.28 (s, 1H, Har), 4.46 (brs, 2H, NH_2), 4.26 (m, 1H, H-5s), 3.86 (s, 3H, OCH_3), 2.84 (m, 1H, H-3x), 2.70 (m, 1H, H-3n), 2.60 (m, 1H, H-6s), 2.55 (m, 1H, H-1), 2.33 (s, 3H, NCH_3), 2.00 (m, 1H, H-7s), 1.91 (brs, 1H, H-4), 1.70 (m, 2H, H-8a, H-8s), 1.50 (m, 1H, H-7a), 1.20 (m, 1H, H-6a). Anal. found: C, 59.41; H, 6,83; N, 13.11. Continued elution gave compound **19**: Yield: 16%; mp 191–194 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 2H, Har, NH amide), 6.27 (s, 1H, Har), 4.33 (brs, 2H, NH_2), 4.22 (m, 1H, H-5a), 3.89 (s, 3H, OCH_3), 2.96 (m, 1H, H-3n), 2.56 (m, 1H, H-1), 2.49 (m, 1H, H-3x), 2.35 (s, 3H, NCH_3), 2.04 (m, 1H, H6a), 1.96 (m, 1H, H-7s), 1.86 (m, 1H, H-4), 1.70 (m, 3H, H-6s, H-8a, H-8s), 1.38 (m, 1H, H-7a). Anal. found: C, 59.02; H, 69.02; N, 13.02.

10. The receptor binding assays have been described in detail elsewhere.[12] **5-HT$_3$ receptor binding** was determined according to Wong et al.[13] A suspension of enthorinal cortex membranes of rat brain (500–600 μg of protein) was incubated at 25 °C for 30 min with 2 nM [^{3}H]-LY278584 in 50 mM Tris–HCl buffer, pH 7.4, containing 5 mmol/l $CaCl_2$ and 0.1% ascorbate, and displacer drug was added. Non-specific binding was determined using 10 μmol/l cold 5-HT. **5-HT$_4$ receptor binding** was determined according to Grossman et al.[14] A suspension of striatum membranes of guinea-pig brain (800–900 μg of protein) was incubated at 25 °C for 30 min with 2 nM [^{3}H]-GR-113808 in 50 mM Hepes buffer, pH 7.4, and displacer drug was added. Non-specific binding was determined using 10 μmol/l cold 5-HT. **D$_2$ receptor binding** was determined according to Kohler et al.[15] A suspension of striatum membranes of rat brain (300–400 μg of protein) was incubated at 25 °C for 60 min with 1 nM [^{3}H]-raclopride in 50 mM Tris–HCl saline buffer, pH 7.7, containing 10 μM pargyline and 0.01% ascorbate, and displacer drug was added. Non-specific binding was determined using 1 μM cold (+)-butaclamol. In all binding assays after the incubation period the reaction was stopped for vacuum filtration (GF/B glass filters) using a Brandel cell harvester and filter-retained radioactivity was measured in a liquid scintillation counter. IC_{50} and K_i values were calculated using the computer program EBDA as described by McPherson.[16]

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