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Synthesis and pharmacological evaluation of the individual stereoisomers of 3-[methyl(1,2,3,4-tetrahydro-2-naphthalenyl)amino]-1-indanone, a potent mast cell stabilising agent

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INSERT GRAPHICAL ABSTRACT HERE

Each stereoisomer of 3-[methyl(1,2,3,4-tetrahydro-2-naphthalenyl)amino]-1-indanone, **1a-d**, was prepared and evaluated *in vitro* for its ability to prevent mediator release induced by different degranulating agents from rodent mast cells and also *in vivo* against passive cutaneous anaphylaxis. The manner in which the stereoisomers prevented direct membrane activation was found to be highly dependent on the stereochemistry of the individual isomers. Stereoisomer **1b** was the most active isomer *in vivo*, exhibiting superior activity to disodium cromoglycate.

Key words: *Mast cells; histamine; allergy.*

Mast cells play a pivotal role in the pathology of allergic disease. Pervading almost all body tissues, mast cells are strategically located on each major organ including the skin, brain, heart, lungs, kidney, and liver.[1] Mast cells are a major source of histamine, a biogenic amine responsible for many of the symptoms of allergy, anaphylaxis and asthma.[2] Regulation of mast cell function would therefore be a significant advance in the treatment of allergic disease. Recent evidence suggests that, in addition to their traditional role in the allergic response, mast cells may also exert many profound effects on a variety of both innate and adaptive immune responses. During autoimmune and inflammatory conditions, mast cells undergo structural changes indicative of secretion without excessive degranulation in a process termed "activation" or "piecemeal" degranulation.[3] Mast cells, given their broad distribution, may therefore play a potentially critical role in a variety of disease states, including multiple sclerosis, rheumatoid arthritis, tumour angiogenesis, cystic fibrosis and irritable bowel syndrome.[4-8] The role of mast cells in angiogenesis and autoimmune conditions opens many possibilities for the development of mast cell directed therapies to treat these conditions.

Previous work by Barlow and Walsh[9] described the synthesis and mast cell stabilising properties of a series of novel tetrahydronaphthalene based compounds. The most active molecule synthesised was the *N*-methylated compound **1**. *In vitro*, this compound displayed potent mast cell stabilising activity when a range of mast cell degranulating agents were employed to stimulate release of pro-inflammatory mediators from rat peritoneal mast cells.

INSERT FIGURE 1 HERE

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Abbreviations: DSCG*:* Disodium cromoglycate; OPA*: ortho*-Phthaldialdehyde; PCA: Passive cutaneous anaphylaxis; PLMC: Porcine lung mast cells; RPMC: Rat peritoneal mast cells.

Using the *in vivo* model of passive cutaneous anaphylaxis (PCA)[10], the activity of this compound was comparable to that of sodium cromoglycate, the most widely prescribed mast cell stabilising agent in clinical use. Nevertheless, the presentation of **1** as a mixture of stereoisomers somewhat compromises its further development. Accordingly, this paper describes the preparation and *in vitro*/*vivo* evaluation of all four stereoisomers of **1**.

Commercially available enantiomers of 2-aminotetralin served as the principal building blocks to prepare the individual stereoisomers **1a-d**. Each enantiomer was coupled to 3-bromo-1-indanone and the resultant pair of diastereoisomers, **2a-b** and **2c-d** from each reaction was purified by a combination of flash column chromatography and preparative TLC. Following their subsequent *N*-methylation with methyl iodide, each stereoisomer of **1**, namely **1a-d**, was furnished (Scheme 1).

INSERT SCHEME 1 HERE

The absolute relative stereochemistry of **2a** was determined by single crystal X ray diffraction.[11] Prior to its analysis, **2a** was first converted into its hydrochloride derivative following treatment with gaseous HCl. Single crystals of **2a.HCl** formed after its dissolution in methanol and gradual evaporation of the solvent on standing at room temperature. The crystal structure obtained (Figure 2) clearly shows the expected *R* configuration for the aminotetralin portion of the molecule, while the new stereogenic centre at C3 is in the *S* form. Interestingly, the tetralin ring of **2a** exhibits a half boat conformation. Previous NMR and X ray studies on 2-aminotetralin derivatives have shown that introduction of an amino substituent alters the symmetry of the tetralin aliphatic ring, producing up to eight different possible conformations.[12, 13] It has been demonstrated that the half boat arrangement of the non aromatic region of 2-aminotetralin derivatives is lower in energy than the half chair conformation.[12, 14-16]

INSERT FIGURE 2 HERE

Stereoisomers **1a-d** inhibited compound 48/80, A23187, concanavalin A and vancomycin induced histamine release from RPMC dose dependently. Slight differences in the activity of each isomer were apparent at 20 μM (Table 1 and Figure 3). However, distinct differences in IC_{50} values were noted. Stereoisomer **1c** exhibited the strongest effect against compound 48/80, concanavalin A and vancomycin induced release while **1b** was the most effective against A23187. Each stereoisomer, at 20 μM, was also evaluated against A23187 and anti IgE mediated release of histamine from porcine lung mast cells (PLMC) (Table 2). In general, at the concentration used, the individual isomers of **1** exhibited superior inhibition against anti IgE that when A23187 was used to stimulate release. Perhaps reflecting species heterogeneity of mast cells, the individual isomers of **1** also displayed weaker mast cell protective effects against A23187 when this secretagogue was used to induce degranulation from RPMC.

INSERT TABLE 1 and FIGURE 3 HERE

INSERT TABLE 2 HERE

INSERT TABLE 3 HERE

In vivo, **1a, c** and **d** inhibited the IgE mediated PCA reaction at comparable levels to disodium cromoglycate ($p<0.05$) (Table 3). As stereoisomer **1b** displayed superior efficacy in comparison to its optical isomers *in vivo* it was used in a dose ranging study. In this study, the protective effect of this compound *in vivo* was also evaluated at 2.0 and 1.0 mg kg^{-1} (Figure 3).

INSERT FIGURE 4 HERE

The mast cell stabilising activity of each stereoisomer of **1** was evaluated *in vitro* and *in vivo* using rodent based models. Cognisant of mast cell heterogeneity between species, their activity *in vitro* was also evaluated using porcine lung mast cells. Compound 48/80, calcium ionophore A23187, vancomycin and concanavalin A were used to stimulate histamine release from RPMC. These secretagogues were selected based on their unique mechanisms of mast cell activation. Compound 48/80 is a hypotensive polymer amine, which causes perturbation of the mast cell membrane resulting in histamine release with similar kinetic characteristics to an antigen[17] while calcium ionophore A23187 facilitates Ca^{2+} influx into cells by forming lipid-soluble complexes, resulting in concomitant release of histamine.[18] Concanavalin A is a plant lectin which interacts with sugar residues located on the Fc region of FcεRI[19] while vancomycin is an antibiotic that is effective against Gram-positive bacteria and is implicated in the pathogenesis of "red man syndrome" due, in part, to the release of histamine from mast cells.[20]

Interestingly, stereoisomers **1a-d** inhibited histamine release stimulated by A23187, indicating that their inhibitory effects are at least partially mediated downstream of FcεRI crosslinking or mast cell surface receptor activation. Furthermore, differential effects were observed in each of the *in vitro* models, reflecting distinct potencies of each isomer. Using RPMC, stereoisomer **1c** was the most effective inhibitor of histamine release stimulated by compound 48/80, concanavalin A and vancomycin, while **1b** displayed the greatest activity against A23187. Significantly less inhibitory activity was observed when A23187 was used to induce release from PLMC in the presence of stereoisomers **1a-d,** presumably due to mast cell heterogeneity between species. Employing PLMC and anti IgE as stimulant, **1b** and **1c** at 20 μM produced almost complete inhibition of histamine release, as shown in Table 2. In the PCA test, the reference compound DSCG at 3 mg kg^{-1} completely abolished the allergic response. Perhaps surprisingly, **1b** was the most active compound in the PCA test, and performed significantly better than **1c** in this assay. This may be accounted for through partial metabolism of **1c** *in vivo*. A dose ranging study on **1b** was also performed *in vivo*. At lower doses of 1 and 2 mg kg^{-1}, **1b** inhibited the response in a dose dependent manner as shown in Figure 3.

It is apparent from the biological data amassed in this study that the manner in which stereoisomers **1a-d** prevent mast cell exocytosis is profoundly affected by variations in the three dimensional arrangement of the molecule. Each releasing agent investigated stimulates mast cell degranulation by a distinct mechanism. The three dimensional arrangement of individual isomers may facilitate greater activity at membrane binding sites, or allow an increased capacity to interrupt signalling pathways.

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Figure captions

Figure 1. Compound 1
Figure 2. View of the molecule **2a** with atomic labeling. Displacement ellipsoids are drawn at the 50% probability level.
Figure 3. Mast cell-stabilising activity of **1a-d** on compound 48/80 (A), A23187 (B), Con A (C) or vancomycin (D)-induced histamine release from RPMC.
Figure 4. Dose dependent inhibition of PCA reaction by stereoisomer **1b**[a].

Scheme caption

Scheme 1. Synthesis of compounds **1a-d** (*a*) Cs_2CO_3, DMF, R.T. 1hr; (*b*) MeI, Cs_2CO_3, DMF, R.T., 1.5hr.

Table 1. Mast cell-stabilising activity of 1a-d in RPMC induced by various secretagogues[a,b]

Compound	48/80		A23187		Con A		Vancomycin	
	% I	IC_{50}	% I	IC_{50}	% I	IC_{50}	% I	IC_{50}
1a	93	3.03	85**	9.48	51	13.13	64	0.01
1b	99*	1.51	69*	4.08	56	16.92	44	4.47
1c	107***	1.49	68**	8.06	54	1.60	79	0.01
1d	105***	2.18	61	6.97	49	1.60	80	12.61

[a]% Inhibition (% I) calculated at a concentration of 20 μM.[b](IC_{50} values are quoted in μM).
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (Tukey-Kramer multiple comparisons test, activities in comparison to **1.** Data represents at least four individual experiments)

Table 2. Mast cell-stabilising activity of 1a-d in PLMC induced by various secretagogues[a]

Compound	% inhibition	
	A23187	anti-IgE
1a	22*	89
1b	31*	53*
1c	40	86
1d	48	81

[a]% Inhibition calculated at a concentration of 20 μM

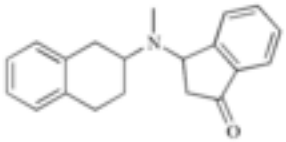
*p<0.05 (Tukey-Kramer multiple comparisons test, activities in comparison to **1**. Data represents at least three individual experiments).

Table 3. PCA inhibitory activity of 1a-d[a]

Compound	% inhibition
1	83
1a	77
1b	111*
1c	73
1d	68
DSCG	73

[a]Tested at a dose of 3 mg kg^{-1}

*$p<0.05$ (Tukey-Kramer multiple comparisons test, activities in comparison to **1**. Data represents at least three individual experiments)



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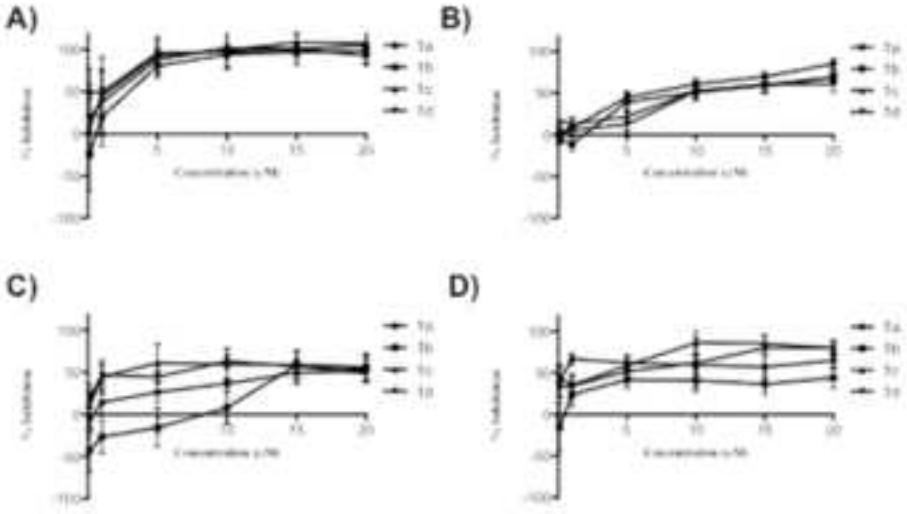
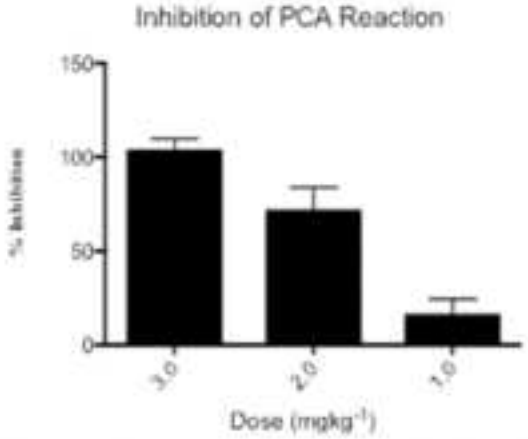
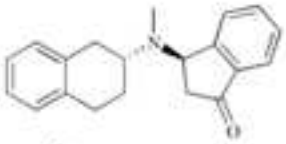
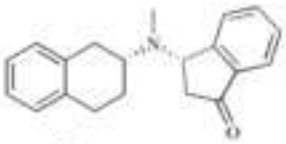




[a]Animals were administered **1b** simultaneously with antigen in 1% DMSO at a dose of 3.0, 2.0, and 1.0 mg kg^{-1}, respectively. Results are expressed as mean inhibition ± S.E.M.

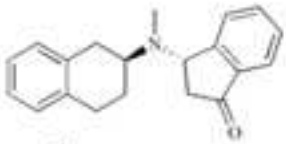


1a

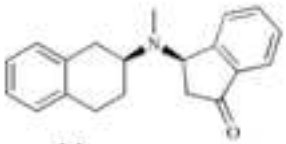


1b

IC_{50} 1.51μM vs. 48/80 in RPMC
53% inhibition of anti IgE in PLMC
Most active isomer *in vivo*
vs. PCA reaction



1c



1d