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Investigation into the mast cell stabilizing activity of nature-identical and synthetic indanones

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Abstract

As part of an ongoing search for novel molecules with therapeutic potential we examined the mediator release inhibition activity of a number of indanones and their derivatives. The aldol condensation product **18** was approximately twice as potent as disodium cromoglycate as an inhibitor of compound 48/80-stimulated histamine release from rat peritoneal mast cells. The activity of this class of dimeric indanone compound is significantly higher than controls and may represent a new class of mast cell stabilizing agents. Compound **18** has been selected for further biological evaluation of its mast cell stabilization profile.

Introduction

In previous reports we established that a broad array of nature-identical and synthetic indanone derivatives demonstrate smooth muscle relaxant activity as measured by the inhibition of calcium-induced contractures of guinea pig ileum in a high potassium, calcium-free medium (Sheridan et al 1990, 1999a, b). The most active of a range of compounds was found to be the nature-identical sesquiterpene pterosin Z (**1**), a tetramethyl indan-1-one with a C-6 hydroxyethyl side-chain (Sheridan et al 1999b) (Figure 1). The level of activity demonstrated by this compound is greater than that reported for the natural indanone onitin (**2**) (1×10^{-4}) (Ho et al 1985; Yang 1985) but less active than the control nifedipine (1×10^{-8}) (Sheridan et al 1999a). From these studies a mechanism of action involving interference with calcium handling in the smooth muscle cell has been proposed for this series of compounds.

In addition to a role in excitation–contraction coupling, many other physiological processes are regulated by calcium. For example, it is well established that calcium plays a role in mast cell exocytosis and mediator release (Foreman & Mongar 1972; Cochrane & Douglas 1974; Cochrane et al 1982; Fasolato et al 1993). The structurally related indanone nivemedone (**3**) (Buckle et al 1973) has been shown to act as a mediator release inhibitor. Because of the wider potential of the pterosin indanone group to affect calcium-mediated processes, the ability of the series to act as mediator release inhibitors was also investigated. During the course of our earlier work a number of synthetic dimeric indane compounds were also synthesized (Farrell 1994). The smooth muscle relaxant and mediator release inhibition activity of this group of compounds is also presented and discussed in this report.

Materials and Methods

Chemistry

All reagents were commercial materials unless otherwise stated. NMR spectra were recorded on a Bruker MSL 300 instrument at 300 MHz. Where distinguishable the values of the minor stereoisomer are italicized. $CDCl_3$ was used as solvent with $SiMe_4$ as internal standard. MS (70 eV, high resolution): AEI MS 30. GC/MS (70 eV): Hewlett Packard 5890 with a 5970 mass selective detector using α-methyl benzene and 2,6-bis(1,1-dimethylethyl)-4-methylphenol as internal standards. IR spectra were recorded on a Nicolet

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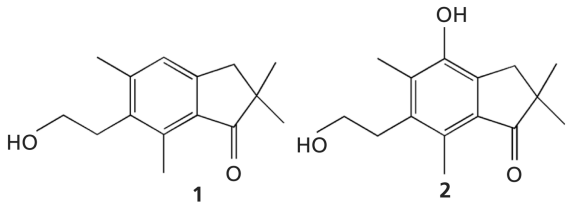
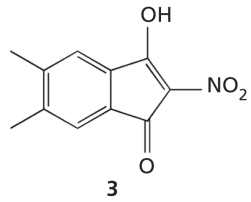
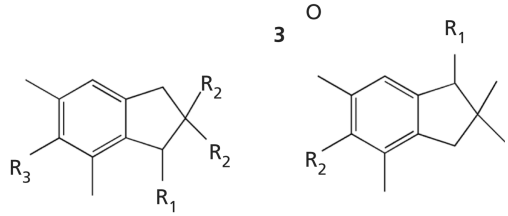
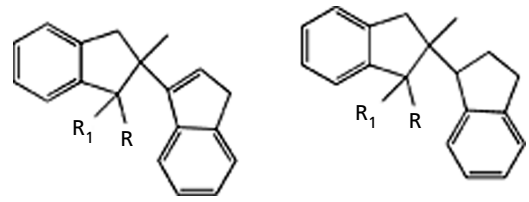






4 R_1 = O, H, R_2 = CH_3 , R_3 = OH
5 R_1 = O, H, R_2 = CH_3, R_3 = OAc
6 R_1 = H, H, R_2 = CH_3, R_3 = $CHCH_2OAc$
7 R_1 = O, R_2 = H, R_3 = $CH{=}CH_2$
8 R_1 = O, R_2 = CH_3, R_3 = $(CH_2)_2COOH$
9 R_1 = H, H, R_2 = CH_3, R_3 = $(CH_2)_2COOCH_3$
10 R_1 = O, R_2 = $CH{=}CHCOOCH_3$
11 R_1 = O, R_2 = $(CH_2)_3OH$
12 R_1 = O, R_2 = $(CH_2)_2COOCH_3$
13 R_1 = O, R_2 = $(CH_2)_2COOH$

Figure 1 Structures of compounds **1** to **13**.



14 R = R_1 = H
15 R = H, R_1 = OH
16 R = R_1 = O
17 R = H, R_1 = OH
18 R = R_1 = H

Figure 2 Structures of compounds **14** to **18**.

205 FT-IR. Melting points were determined on a hot-stage apparatus and are uncorrected. TLCs were run on commercially pre-coated plates (Kieselgel 60F_{254}; Merck). Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM; Merck).

The syntheses of **4**–**13** are outlined in Sheridan et al (1999a) (Figure 1) and the structures of compounds **14** to **18** are shown in Figure 2.

*2-(1-ind-1-enyl)-2-methylindan-1-one (**14**)*
A solution of iodomethane (4.72 mL, 75 mmol) in ether (50 mL) and a solution of potassium tert-butoxide (8.49 g, 75 mmol) in tert-butanol (150 mL) were added simultaneously to a solution of indan-1-one (10 g, 75 mmol) in ether (100 mL) and tert-butanol (20 mL). The reaction mixture was stirred at reflux for 2 h. The solution was brought to room temperature and partitioned between equal volumes of ethyl acetate and aqueous ammonium chloride (1:1, 300 mL). The organic layer was removed, dried over sodium sulfate and the solvent filtered and evaporated to yield a crude oil that was purified by flash column chromatography (eluant pet. ether:EtOAc 9:1) to yield a white solid (28%): mp 112–113°C; IR (KBr) 2361, 1715, 1606, 1759 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 1.67 (3H, s, CH_3), 3.20 (1H, d, J = 17 Hz, CH), 3.40 (2H, d, J = 2 Hz, CH), 3.69 (1H, d, J = 17 Hz, CH), 6.52 (1H, t, J = 2 Hz, CH), 6.87 (1H, d, J = 8 Hz, Ar–H), 7.15 (2H, m, 2 × Ar–H), 7.48 (3H, m, 3 × Ar–H), 7.68 (1H, m, Ar–H), 7.92 (1H, d, J = 8 Hz, Ar–H). ^{13}C NMR (75.47 MHz, $CDCl_3$): 23.9, 37.6, 41.2, 50.5, 119.8, 124.1, 124.6, 124.8, 125.9, 126.8, 127.7, 130.1, 135.2, 135.6, 143.0,144.9, 145.8, 152.3, 208.6. MS: M^+ 260.1183 (90) (requires 260.1197).

*2-(1-ind-1-enyl)-2-methylindan-1-ol (**15**)*
Compound **14** (1.4 g, 5.4 mmol) was dissolved in EtOH (20 mL). $NaBH_4$ (250 mg, 6.6 mmol) was added to the reaction in small portions over 10 min and the reaction was stirred at 0°C for 1 h. The reaction was brought to room temperature and stirred for a further 3 h. The reaction mixture was poured onto iced water (20 mL) and extracted into Et_2O (3 × 20 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the residue was purified by flash column chromatography (eluant pet. ether:EtOAc 98:2). The alcohol **15** was recovered as an oil (1.26 g, 89%). IR (KBr) 3429 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 1.43 (6H, bs, J = 6 Hz, 2 × CH_3), 3.01 (1H, d, J = 15 Hz, CH), 3.11 (1H, d, J = 15.9 Hz, CH of CH_2), 3.43 (2H, s, CH_2), 3.48 (2H, s, CH_2), 3.53 (1H, d, J = 16 Hz, CH), 3.83 (1H, d, J = 15 Hz, CH), 5.40 (1H, s, CH), 5.66 (1H, s, CH), 6.49 (1H, t, J = 2 Hz, CH), 6.52 (1H, t, J = 2 Hz, CH), 7.27–7.46 (5H, m, Ar–H), 7.58 (2H, t, J = 5 Hz, Ar–H), 7.76 (1H, d, J = 7 Hz, Ar–H). ^{13}C NMR (75.47 MHz, $CDCl_3$): 19.8, 25.5, 37.6, *37.9*, 43.1, *43.2*, 49.9, *50.2*, 80.7, *81.6*, 121.0, 121.5, 124.2, 124.3, 124.7, 125.1, 125.3, 125.7, 126.1, 126.3, 126.8, 128.0, 128.6, 128.8, 130.8, 140.2, 142.7, 143.1, 143.7, 143.9, 145.4, 148.0, 150.0. MS: M^+ 262.1360 (68), $C_{19}H_{18}O$ (requires 262.1353).

*2-(1-indanyl)-2-methylindan-1-one (**16**)*
Compound **14** (1 g, 3.85 mmol) was dissolved in EtOH (20 mL) and EtOAc (10 mL). 10% Pd/C (catalytic) was added to this solution and the reaction was stirred under an atmosphere of H_2 for 2 h. The catalyst was removed by filtration and the solvent was evaporated to dryness to yield **16** as an oil, which was crystallized from Et_2O as a white solid mp 89–90°C (0.76 g, 76.34%). IR (KBr) 1709, 1606 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 1.46 (3H, s, CH_3), 1.45–2.30 (2H, 4 × m, CH_2), 2.68, 2.98 (2H, dd,

$J = 2$, 17.8 Hz, CH_2), 2.79 (2H, m, CH_2), 3.65 (1H, m, CH), *3.84 (1H, m, CH)*, 6.73 (1H, bm, Ar–H), *6.99 (1H, bm, ArH)*, 7.30 (5H, bm, Ar–H), 7.56 (1H, m, Ar–H), 7.78 (1H, m, $3 \times$ Ar–H), *7.83 (1H, m, $3 \times$ Ar–H)*. ^{13}C NMR (75.47 MHz, $CDCl_3$): 24.4, *24.6*, 29.0, *28.3*, 31.8, *31.2*, 37.6, *36.8*, 50.7, *50.6*, 52.9, *52.6*, 124.2, 124.8, 125.5, 125.9, 126.4, 126.8, 127.4, 134.8, 136.2, 144.2, 145.0, 153.4, 210.9, *211.0*. Analysis of $C_{19}H_{18}O$ requires C 85.49%, H 6.87%; found C 85.59%, H 6.45%. MS: M^+ 262.

*2-(1-indanyl)-2-methylindan-1-ol (**17**)*
Compound **16** (229 mg, 0.87 mmol) was dissolved in EtOH (10 mL). $NaBH_4$ (0.03 g, 1.0 mmol) was added to the reaction in small portions over 10 min and the reaction was stirred at 0°C for 1 h. The reaction was brought to room temperature and stirred for a further 3 h. The reaction mixture was poured onto iced water (20 mL) and extracted into Et_2O (3×20 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the residue purified by flash column chromatography (eluant pet. ether:EtOAc 98:2) to yield **17** as an oil (176 mg, 76%). IR (KBr) 3390 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 0.86 (3H, s, $\underline{CH_3}$), *0.94 (3H, $2 \times$ s, CH_3)*, 2.41 (6H, bm, $\underline{CH_2}$), 5.19 (1H, bs, $\underline{CH}$OH), *5.23 (1H, bs, CHOH)*, 7.29 (8H, bm, Ar–H). ^{13}C NMR (75.47 MHz, $CDCl_3$): 15.1, *16.0*, 27.9, *28.9*, 31.9, *32.1*, 43.6, *43.8*, 52.1, 54.5, *54.1*, 82.5, *82.0*, 123.7, 124.7, 124.9, 125.3, 125.5, 125.8, 125.9, 127.8, 140.2, 144.0, 144.7, 145.2. MS: M^+ 264.

*2-(1-indanyl)-2-methylindane (**18**)*
Compound **14** (100 mg, 0.38 mmol) was dissolved in EtOH (5 mL) and EtOAc (1 mL). To this solution HCl (2 mL, 37%) was added with water (0.4 mL) and 10% Pd/C (catalytic) and the reaction was stirred under an atmosphere of H_2 for 2 h. The catalyst was removed by filtration and the product was extracted into EtOAc (2×20 mL), which was washed with H_2O (2×20 mL). The organic solvent was dried over $MgSO_4$, filtered and evaporated to dryness to yield **18** as an oil (0.84 g, 89.14%). IR (KBr) 1603 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 1.52 (3H, s, CH_3), 2.14 (2H, m, CH$\underline{CH_2}CH_2$), 2.80 (2H, d, $J = 15.5$ Hz, C$\underline{CH_2}$), 3.04 (2H, d, $J = 15.5$ Hz, C$\underline{CH_2}$), 3.11 (2H, m, CHCH$_2\underline{CH_2}$), 3.49 (1H, m, $\underline{CH}CH_2CH_2$), 7.26 (8H, bm, Ar–H). ^{13}C NMR (75.47 MHz, $CDCl_3$): 22.6 (CH_3), *28.6*, 31.9, *46.3*, 46.5 (CH_2s), 55.5 (CH), 124.5, 124.7, 125.2, 125.8, 125.9, 126.4 (ArCHs), 144.5, 143.0, 145.1, 145.2 (Ar–Cs). Analysis of $C_{19}H_{20}$ requires C 96.77%, H 3.22%; found C 95.84%, H 3.05%. MS: M^+ 248.

Peritoneal mast cell assay

Female Wistar rats (200–300 g) were killed in an atmosphere of saturated CO_2. Pre-warmed buffered salt solution (BSS) (NaCl 137 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 0.5 mM, NaH_2PO_4 0.4 mM, glucose 5.6 mM, HEPES 10 mM; 10 mL) was injected i.p. and the abdomen was massaged for 3 min. The BSS, with suspended mast cells and other cells, was aspirated following a mid-line incision. The aspirate was centrifuged for 5 min at 400 *g* and the supernatant removed. The cells were resuspended in 4.5 mL BSS at 4°C and centrifuged as before. The cells were washed in this manner a total of three times. Following the final wash, the pelleted cells were stored at 4°C for use as soon as possible. The cells were resuspended in 7 mL BSS. From this, 0.5 mL aliquots were transferred to each of the incubation tubes. In addition to each compound replicate, sample tubes were also prepared for basal histamine release, total histamine content, compound 48/80-stimulated release and solvent control. The solvent blank was supplemented with 0.5% (v/v) DMSO or 0.5% (v/v) distilled water. The two positive controls were supplemented with 0.5% (v/v) distilled water/2×10^{-5} M disodium cromoglycate (DSCG) and 0.5% (v/v) DMSO/2×10^{-5} M DSCG. The test compound incubation tubes contained 2×10^{-5} M test compound/0.5% (v/v) DMSO. After 10 min at 37°C, with gentle agitation, compound 48/80 was added to a final concentration of 2 mg mL^{-1} in order to stimulate histamine release. The cell stimulation was stopped after 2 min by the addition of 0.5 mL ice-cold BSS and the incubation tubes were transferred to an ice bath. The cell suspensions were centrifuged for 5 min at 400 *g*. One tube was placed at 100°C for 2 min prior to centrifugation in order to measure the total histamine content of the mast cells. The supernatants were retained for histamine assay.

Supernatant (2 mL from each tube) was added to NaOH (1 M, 0.4 mL) and o-phthalaldehyde (1% (w/v) in methanol, 0.1 mL). This was incubated at room temperature for 4 min. The reaction was stopped by the addition of HCl (3 M, 0.2 mL). The supernatant from each incubation tube was assayed in duplicate and run simultaneously with a standard curve in the range 0–1000 ng mL^{-1}. The presence of the fluorescent product of the reaction was measured using a Shimadzu RF-1501 spectrofluorophotometer set at $\lambda_{ex} = 360$ nm, $\lambda_{em} = 450$ nm. Values are expressed as mean ± s.e.m. and statistical comparisons were made using the Kruskal–Wallis test followed by a post test using Dunn's multiple comparison test, with significance taken at the $P < 0.05$ level.

Animals were sacrificed according to guidelines laid down by the working party report (Laboratory Animals (1996) 30, 293-316, Laboratory Animals (1997) 31, 1–32) on Directive 86/609/EEC (No. L 358, ISSN 0378-6978), which is endorsed by the Bioresources Ethical Review Committee of the university.

Results and Discussion

The effect of active synthetic compounds (ED50 greater than 1×10^{-5}) from initial studies (Sheridan et al 1999a) was evaluated on the secretion of histamine from mast cells using the anti-allergenic drug sodium cromoglycate as internal standard. For each compound replicate, values are expressed as a percentage inhibition of compound 48/80-induced histamine release compared to the vehicle control, on the same cell sample. The basal histamine release in untreated cells was expressed as a percentage of the total histamine content of the cells in suspension. Overall, the mean basal release was (9.60 ± 1.02%) of total histamine content of the cells (n = 55). The maximum stimulated

histamine release by compound 48/80 was $67.38 \pm 2.90\%$) in the presence of 0.5% (v/v) distilled water and $54.87 \pm 2.69\%$ in the presence of 0.5% (v/v) DMSO of total histamine content of the cells ($n = 55$). Mean inhibition with DSCG was $10.5 \pm 1.1\%$, $n = 55$.

The compounds are divided into two distinct groups: monomeric (**1**–**13**) and dimeric compounds (**14**–**18**). The range of mast cell stabilizing activity demonstrated by these structurally varied groups proved considerable. The first group of compounds investigated were C-6 derivatives of the basic pterosin nucleus (**4**, **5**, **6** and **7**). As a group these compounds inhibited mast cell histamine release as effectively as sodium cromoglycate. Phenol **4** inhibited histamine release by $10.4 \pm 2.9\%$ compared with the internal standard (10.5 ± 1.1) at a concentration of $5\,\mu g\,mL^{-1}$. Conversion of **4** to its acetate **5** led to a reduction in mast cell histamine release to a similar level to that of sodium cromoglycate. Synthetic pterosin Z (**1**), which has a two-carbon side-chain at the C-6 position, was also active ($1.3 \pm 0.3 \times 10^{-6}$ M); derivatization to its 6-acetoxyl derivative **6** resulted in a decrease in activity ($4.2 \pm 0.6 \times 10^{-6}$ M). The most potent inhibitor of mast cell histamine release from this group of pterosin analogues proved to be the synthetic vinyl indanone derivative **7** ($7.2 \pm 1.7\%$) (Table 1).

Pterosin and isopterosin derivatives with three-carbon side-chains at C-6 (C-5) also demonstrated mast cell stabilization activity similar to that of the positive control. These compounds included the methyl propenoate, methyl propanoate and hydroxyl propyl derivatives **8**–**11**. The greatest activity was shown by the C-6 methyl propanoate analogue **8** ($12 \pm 7.3\%$). The propanoic acid derivative **12** showed minimal activity. Diol **13** also demonstrated activity equipotent with the positive control ($11.5 \pm 5.2\%$) and only slightly less potent than **8**. Again all components were tested at $5\,\mu g\,mL^{-1}$. Conversion of **8** to its derivative **9** resulted in a similar level of activity ($10.2 \pm 5.7\%$). The pterosin analogue **10** was, in contrast, found to increase histamine release ($8.7 \pm 11.5\%$). Reduction to the propanol **11** resulted in a compound with a similar level of activity ($8.3 \pm 1.4\%$) to sodium chromoglycate.

A series of aldol condensation products formed as by-products during this study were also screened for mast cell stabilization activity. This series of compounds proved to be the most active of all, with **14** being equipotent ($15.3 \pm 2.2\%$) with the positive control. The racemic indanol **15** had similar activity. Because of this unexpected activity the related compounds **16**–**18** were also synthesized and the activity was measured (Table 1, Figure 3). Compounds **16** and **17** were also equipotent with DSCG. Compound **18** showed a significantly ($P < 0.05$) greater inhibition of histamine release when compared to DSCG (see Figure 3). Compound **18** ($23.0 \pm 2.2\%$) was approximately twice as potent as DSCG as an inhibitor of compound 48/80-stimulated histamine release from rat peritoneal mast cells.

We have previously shown (Sheridan et al 1999a, b) that (similar) compounds can inhibit calcium contractions of intestinal smooth muscle, although it is not known at this time whether this effect is due to an effect on voltage-operated calcium channels or on the intracellular processes subsequent to either calcium entry or release from intracellular binding sites. Such processes may include interference with calcium–calmodulin binding or the subsequent reactions in the calmodulin cascade. In secretory

Table 1 Effect of various compounds (2×10^{-5} M) and DSCG (2×10^{-5} M) ($n = 55$) on the % inhibition of compound 48/80-stimulated histamine release (2×10^{-5} M) from rat peritoneal mast cells

Compound	% Inhibition of histamine release	s.e.m.	n
1	8.7	5.3	4
4	10.4	2.9	4
5	7.2	5.2	4
6	1.3	6.7	4
7	9.5	6.5	4
8	11.9	7.3	4
9	10.2	5.8	4
10	−8.8	11.5	4
11	8.3	1.4	4
12	1.9	2	4
13	11.5	5.2	4
DSCG	10.5	1.1	55

Mean ± s.e.m., $n = 4$; DSCG; $n = 55$.

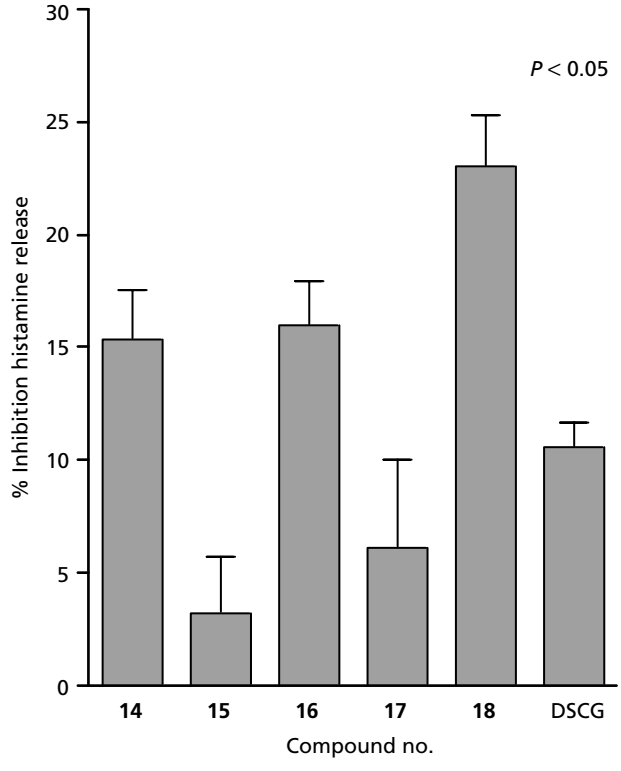


Figure 3 Effect of compounds **14–18** (2×10^{-5} M) and DSCG (2×10^{-5} M) ($n = 55$) on the percentage inhibition of compound 48/80-stimulated histamine release (2×10^{-5} M) from rat peritoneal mast cells. Mean ± s.e.m., $n = 4$.

cells, calcium also plays a role in their function and these compounds may interfere in the process to inhibit mediator release.

Conclusion

The structures of the aldol condensation products differ significantly from the pterosins and other monomeric indanones investigated. The activity of this class of compounds is significantly higher than controls and may represent a new class of mast cell stabilizing agents.

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