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Original article

A series of 1, 2-coupled indane dimers with mast cell stabilisation and smooth muscle relaxation properties

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ABSTRACT

Asthma is characterised by bronchoconstriction and inflammation, with infiltration and activation of inflammatory cells such as eosinophils and mast cells, and subsequent release of inflammatory mediators. Much of the therapy directed at the treatment of asthma is either to provide symptomatic relief through bronchodilation or to reduce inflammation to prevent or delay airway remodelling. In an attempt to address both of these issues, a novel series of 1,2-indane dimers has been synthesized and evaluated for smooth muscle relaxant and mast cell stabilising activities. We have identified two lead compounds, **5** and **15**, which have substantial mast cell stabilisation activity.

1. Introduction

Calcium plays a key role, not only in excitation-contraction coupling in smooth muscle, but is intimately involved in the release of mediators from secretory cells such as mast cells. For example, ceramide kinase (CERK) is a calcium/calmodulin-dependant enzyme thought to play an important role in mast cell function [1]. Phosphorylation of the sphingolipid metabolite, ceramide by CERK produces C1P, which has been suggested as an important mediator of mast cell degranulation [2,3]. Inhibition of CERK can suppress IgE-mediated mast cell degranulation and small-molecule CERK inhibitors have been suggested as potential therapeutic agents with application in the treatment of allergic diseases such as asthma [4].

Furthermore, calcium channel blockers, such as nicardipine, not only may be expected to have a beneficial effect in asthma as a consequence of its relaxant effect on bronchial smooth muscle, but it has also been shown to inhibit the release of Th2-type cytokines, such as IL4, IL5 and IL13 from T-lymphocytes; such cytokine release is calcium-dependant and these cytokines are thought to play an important role in asthma [5].

The smooth muscle relaxant and anti-allergenic properties of simple indanes and indanones has been reported for natural [6] and synthetic indane compounds [7]. In our earlier studies we have established that a group of synthetic monomeric indanones related to the pterosin family of natural products exhibit significant smooth muscle relaxant activity, by an inhibition of a calcium or calcium/calmodulin-dependant mechanism [8–12]. More recently we have observed a similar effect in a number of synthetic dimeric indane by-products. These compounds also showed significant inhibition of compound 48/80-stimulated histamine release from rat peritoneal mast cells [13,14]. We now present data on a series of related 1,2 -coupled indane dimers which are significant inhibitors of histamine release and have moderate smooth muscle relaxing activity.

2. Chemistry

The target compounds in this study were 1, 2-coupled indane dimers **3–15**. We recently prepared compound **2** by base catalysed condensation during the methylation of bromoindan-1-one [13]. In this current study we have developed a versatile synthesis for the 2-alkylated, 1, 2-coupled dimeric ketones using compound **1** as a key intermediate. This compound was prepared in high yield by the base catalysed condensation of indan-1-one. The lithium enolate **2** was readily formed by reaction of **1** with LDA at −78 °C. The enolate was then reacted, without isolation, with a range of alkyl halides (Scheme 1) to yield aliphatic (**3, 4, 6, 7** and **12**) and aromatic derivatives (**9–11**). Compounds **5** and **8** were prepared by Wilkinson's reduction of unsaturated alkyl derivatives **6** and **7** prepared by direct alkylation of **1**. The corresponding alcohols were prepared by sodium borohydride reduction of a methanolic solution of the

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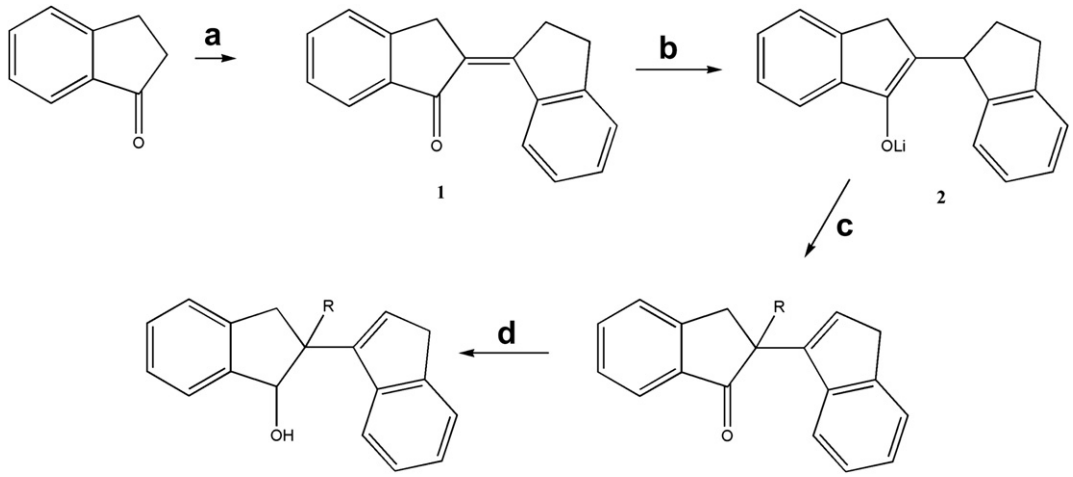

Scheme 1. a) ^{t}BuOK, ^{t}BuOH, Et_2O, rt; b) LDA/THF, −78 °C; c) R-Hal; d) $NaBH_4$/MeOH.

ketones. The diastereoisomeric alcohols were not separated and the mixture of isomers was incorporated into the pharmacological screens. Integration of the CHOH resonances in the ^{1}H NMR spectra of the diastereoisomeric alcohols established the ratio of the composite diastereoisomers and where possible the NMR data for the minor diastereoisomer is reported.

3. Pharmacology

3.1. Smooth muscle relaxant activity

Smooth muscle relaxant activity was assessed on inhibition of calcium-stimulated (2.5 mM) contractures of isolated guinea-pig ileum suspended in high-potassium (45 mM), calcium-free Kreb's solution, as described previously [9]. The results are shown in Table 1. Compound **13** was evaluated in a dose response study. The IC_{50} values are shown in Fig. 1.

3.2. Mast cell stabilisation

Mast cell stabilising activity was assessed on inhibition of histamine release from Compound 48/80-stimulated rat peritoneal mast cells, as described previously [13]. Peritoneal lavage exudates were not purified.

4. Results

Calcium (2.5 mM) caused a sustained contraction of guinea-pig ileum suspended in high-potassium, calcium-free Kreb's solution. Addition of compounds (1×10^{-5} M) **3–10** and **12–15** caused an inhibition of the calcium contraction ranging from less than 20% for compounds **7** and **9** to almost 80% inhibition for **14.** Compound **11** was without smooth muscle relaxant activity. In comparison, nifedipine at 1×10^{-8} M inhibited contractions by 48% (Table 1). A subsequent dose response curve to both nifedipine ($1 \times 10^{-10} - 1 \times 10^{-7}$ M) and compound **14** ($3 \times 10^{-8} - 1 \times 10^{-5}$ M) demonstrated good concentration-effect relationships and resulted in calculated IC_{50} values of $1.47 \pm 0.14 \times 10^{-8}$ M and $6.2 \pm 0.47 \times 10^{-6}$ M respectively (Fig. 1).

Incubation of mast cells with disodium cromoglycate or compounds **3–15** had no effect on basal levels of histamine release, from rat peritoneal mast cells. With the exception of **8**, compounds were shown to have an effect on compound 48/80-stimulated histamine release. Compounds **3–7**, **9**, **11**- **12** and **15** (2×10^{-5} M) inhibited histamine release to a significantly ($p < 0.05$) greater extent than disodium cromoglycate (2×10^{-5} M), with **15** being the most potent, causing an inhibition of histamine release of $49.7 \pm 5.3\%$. In comparison, disodium cromoglycate inhibited histamine release by $9 \pm 0.9\%$ (Table 1). In contrast, compounds **8**, **10**, **13** and **14** potentiated 48/80-stimulated histamine release, with values of 27.8 ± 7.7, $26 \pm 4.8\%$ and $35.5 \pm 6.3\%$ respectively (Table 1).

5. Discussion

We have previously reported that the naturally occurring monomeric indane, pterosin Z (**16**) had smooth muscle relaxant activity against calcium contractions, with an IC_{50} value of 1.3×10^{-6} M. [10]. In comparison, in this study the most potent of the compounds studied was **14** (IC_{50} value of 6.2×10^{-6} M), this being almost equipotent with **16** and compounds **3–6** which were approximately equipotent with **14** and **16** at a single dose of 1×10^{-5} M (Table 1). However, this activity is more than two orders of magnitude less potent than nifedipine. Such limited smooth muscle relaxant activity was disappointing and is unlikely to have any therapeutic potential.

Table 1
Effect of compounds **3**–**15** (1×10^{-5} M) on inhibition of calcium (2.5 mM) contractures of guinea-pig ileum suspended in high-potassium (45 mM), calcium-free modified Kreb's solution and on of Compound 48/80-stimulated (2 μg/ml) histamine release from rat peritoneal mast cells. Values are expressed as a mean ± SEM, expressed as a percentage of stimulated values, *n* as indicated. The effect of nifedipine (1×10^{-8} M) on inhibition of calcium contractures and of disodium cromoglycate (DSCG) (2×10^{-5} M) on histamine release are shown as a comparison.

Compound	Smooth muscle relaxation			Inhibition of histamine release		
	Mean	SEM	*n*	Mean	SEM	*n*
3	41.3	3.8	8	15.6	3.2	5
4	49.6	1.7	6	19.4	2.0	5
5	51.7	3.7	6	41.2	3.6	5
6	51.3	2.0	6	16.6	1.7	5
7	19.9	1.4	6	32.5	3.1	5
8	18.0	2.9	6	−3.2	12.0	5
9	18.9	3.2	7	24.7	3.2	5
10	31.5	2.5	6	−27.8	7.7	5
11	1.8	4.1	8	10.3	2.3	5
12	20.5	2.7	6	21.9	4.4	5
13	34.3	4.8	12	−26.0	4.8	10
14	79.5	2.6	6	−35.5	6.3	5
15	25.6	3.1	12	49.7	5.3	10
Nifedipine	48.1	2.3	6			
DSCG				9.1	0.9	20

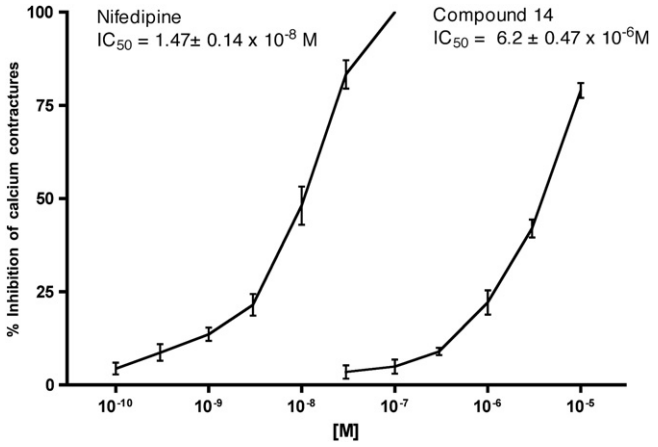

Fig. 1. Effect of compound **13** ($3 \times 10^{-8} - 3 \times 10^{-5}$ M) and Nifedipine, ($1 \times 10^{-10} - 1 \times 10^{-7}$ M), (added cumulatively) on inhibition of calcium (2.5 mM) contractions of guinea-pig ileum suspended in high-potassium (45 mM), calcium-free modified Kreb's solution. Values are expressed as a mean ± SEM, $n = 6$.

Compounds **5**, **7** and **15** were particularly potent as mast cell stabilisers in comparison with disodium cromoglycate (Table 1). Compounds **5** and **15** are more than twice as potent (41.2 ± 3.6 and 49.7 ± 5.3 respectively) as **17** (23.0 ± 2.2), the lead 1,2 coupled dimeric indane identified in our earlier study [13]. Compound **5** most effectively combines smooth muscle relaxant and mast cell stabilisation activity However, the observation that the smooth muscle relaxant activity of ketone **5** is increased by more than 50% on conversion to the corresponding alcohol **14** is not reflected in mast cell stabilisation activity; indeed the converse is true and we observed a potentiation of histamine release (Table 1). It would appear that there is no structure activity relationship in respect of both smooth muscle relaxant and mast cell stabilisation activities: there is no correlation between the potencies of the compounds on inhibition of calcium contractures of guinea-pig ileum and their activity on inhibition of histamine release ($r^2 = 0.078$, $P = 0.33$). A mechanism by which compounds might potentiate compound 48/80-stimulated histamine release in the absence of any additive direct effect is not obvious.

6. Conclusion

The purpose of the study was to determine if it was possible to synthesise a 1, 2 coupled indane dimer which would combine both significant smooth muscle relaxant and mast cell stabilising activities. It was envisioned that the combination of anti-allergic and bronchodilator activities in the same molecule would yield a potential new treatment for asthma. The original hypothesis was that the compounds under investigation interfered in some way with an element of calcium handling that was shared in both excitation-contraction coupling and excitation-secretion coupling. This hypothesis was based on the fact that the calcium/calmodulin complex and its activation of myosin light chain kinase is a key factor in both processes [1,15]. However, the lack of correlation between smooth muscle relaxant and mast cell stabilising activities and the inverse relationship in compound **14** between the two types of pharmacological activity suggests that this is not the case. Despite the inverse relationship in activities observed for compound **14,** the ability of some of the other compounds, particularly compounds **5** and **15**, to inhibit histamine release is significant and may have therapeutic application in the areas of allergy and inflammation.

7. Experimental protocols

7.1. Chemistry

7.1.1. Synthesis of (Z)-2, 3-dihydro-2-(1, 2-dihydroinden-3-ylidene)-inden-1-one (*1*)

Indan-1-one (5 g, 37 mmol) and toluene (80 ml) were placed in a 250 ml round bottomed flask and the solution was dried by azeotropic distillation. To this solution was added Aluminium tri-*tert*-butoxide (4.7 g, 19 mmol) and the reaction mixture was refluxed 60 min. Additional Aluminium tri-*tert*-butoxide (2.3 g, 9.0 mmol) was added after 1 hr. The reaction mixture was cooled poured onto ice water and the product was extracted into Et_2O, dried and evaporated and the residue was purified by flash column chromatography (eluent: petroleum ether: Et_2O; 9:1). After evaporation **1** was recovered as a yellow crystalline solid (48%), m.p. 112–114 °C. Found M^+ 246 ($C_{18}H_{14}O$ requires 246.30). υ_{max} (KBr) 2361.2, 1715.1, 1606.9, 1459.7 cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 3.11 (2H, t, J = 6 Hz, CH_2), 3.54 (2H, m, CH_2), 3.98 (2H, s, CH_2), 7.53 (6H, m, 6 × Ar-H), 7.79 (2H, m, 2 × Ar-H); $^1\delta_C$ ($CDCl_3$, 75.47 MHz): 30.9, 31.5, 33.0, 123.5, 125.7, 125.9,125.9, 126.2, 126.8, 127.2, 130.4, 133.5, 139.5, 140.8, 148.7, 151.7, 154.9, 195.1.

7.1.2. General procedures for synthesis of compounds *3–15*

7.1.2.1. Lithium diisopropylamide (LDA) alkylation reaction. A dry three necked (100 ml) round bottomed flask, under nitrogen, was charged with a solution of dimer in dry THF. The solution was cooled to −78 °C and lithium diisopropylamide (LDA) in THF/heptane/ethylbenzene was added. The solution was stirred at −78 °C for 10 min and the desired organic halide was added. After 15 min the solution was warmed to room temperature and stirred for 3 hr. The solution was poured carefully on ice water and aqueous ammonium chloride (10%) was added. The mixture was extracted with Et_2O (x3). The combined organic extracts were dried over sodium sulphate and evaporated. The crude product was purified by flash column chromatography.

*7.1.2.2. 2, 3-dihydro-2-(1H-inden-3-yl)-2-methylinden-1-one (**3**).* Prepared from (**1**) by a LDA based alkylation. White solid (80%); m.p. 112–114 °C. Found M^+260 ($C_{19}H_{16}$ requires 260.32). υ_{max} (KBr) 2361.2, 1715.1, 1606.9, 1459.7 cm^{-1}. δ_H ($CDCl_3$, 300 MHz): 1.67 (3H, s, CH_3), 3.20 (1H, d, J = 17 Hz, CH), 3.40 (2H, d, J = 2 Hz, CH_2), 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). δ_C ($CDCl_3$, 75.47 MHz): 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. Analysis of $C_{19}H_{16}O$ requires C, 87.69% and H, 6.15%. Found: C, 87.54% and H, 6.25%.

*7.1.2.3. 2, 3-dihydro-2-(1H-inden-3-yl)-2-ethylinden-1-one (**4**).* Prepared from (**1**) by a LDA based alkylation. The reaction yield of (**4**) was 77%. Found M^+274 ($C_{20}H_{18}O$ required M^+274). δ_H (CDCl3, 300 MHz): 0.83 (3H, t, J 8 Hz, CH_3), 2.21 (2H, m, J = 8 Hz, CH_2), 3.34 (1H, d, CH), 3.35 (2H,s, CH_2), 3.59 (1H, d, J = 17 Hz, CH) 6.49 (1H, t, J = 2 Hz, CH), 7.15–8.0 (8H, m, 8 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 8.6, 29.5, 37.4, 38.3, 54.4, 120.1, 123.9, 123.9, 124.4, 125.7, 126.2, 127.3, 129.7, 134.8, 136.9, 143.2, 144.8, 145.1, 152.6, 207.7.

*7.1.2.4. 2, 3-dihydro-2-(1H-inden-3-yl)-2-propylinden-1-one (**6**).* Prepared from (**1**) by a LDA based alkylation. The reaction yield for (**6**) was 67%. Found M^+286 ($C_{21}H_{18}O$ requires 286.14). δ_H ($CDCl_3$, 300 MHz): 2.94 (2H, d, $C\underline{H}_2CH = CH_2$), 3.38 (2H, br s, $C = CHC\underline{H}_2$), 3.53 (2H, ab q, J = 17.5 Hz, $C\underline{H}_2$), 4.99 (1H, dd, J = 1 Hz, 10 Hz, $CH_2CH = C\underline{H}_2$), 5.16 (1H, dd, J = 3.3 Hz, 17 Hz, $CH_2CH = C\underline{H}_2$), 5.62 (1H, m, $CH_2\underline{C}H = C\underline{H}_2$), 6.52 (1H, t, J = 2 Hz, $C = C\underline{H}CH_2$), 7.06 (1H, m,

1 × Ar-H), 7.18 (2H, m, 2 × Ar-H), 7.46 (3H, m, 3 × Ar-H), 7.65 (1H, dt, J = 1.3 Hz & J = 7.6 Hz 1 × Ar-H), 7.87 (1H, d, J = 7.5 Hz, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 37.6, 37.6, 41.1, 53.9, 118.7, 120.2, 124.0, 124.2, 124.6, 125.8, 126.4, 127.6, 130.3, 132.9, 135.2, 136.2, 143.0, 144.8, 144.9, 152.7, 207.4.

*7.1.2.5. 2, 3-dihydro-2-(1H-inden-3-yl)-2-((E)-pent-2-enyl)-inden-1-one (**3**).* Prepared from (**1**) by a LDA based alkylation as outlined. The reaction yield for (**7**) was 40%. Found M^+316 ($C_{21}H_{18}O$ requires 316.43).δ_H ($CDCl_3$, 300 MHz): 0.73 (3H, t, CH_2CH_3), 1.83 (2 H, m, CH_2CH_3), 2.85 (2H, d, $CH_2CH=CH$), 3.8 (2H, br s, $C=CHCH_2$), 3.50 (2H, ab q, J = 13.0 Hz, $COCCH_2$), 5.18 & 5.58 (2H, 2 × m, $CH_2CH=CHCH_2$), 6.52 (1H, t, J = 2 Hz, 1 × $C=CHCH_2$), 7.01 (1H, m, 1 × Ar-H), 7.15 (2H, m, 2 × Ar-H), 7.40 (3H, m, 3 × Ar-H), 7.65 (1H, t, 1 × Ar-H), 7.85 (1H, d, 1 × Ar-H). δ_C ($CDCl_3$, 300 MHz): 13.4, 25.4, 37.6, 37.6, 39.9, 54.3, 120.1, 123.1, 124.0, 124.0, 124.6, 125.8, 126.3, 127.4, 130.2, 135.0, 136.6, 136.9, 143.2, 144.9, 145.1, 152.9, 207.9.

*7.1.2.6. 2-benzyl-2, 3-dihydro-2-(1H-inden-3-yl)-inden-1-one (**9**).* Prepared from (**1**) by a LDA based alkylation with benzyl bromide. The reaction yield for (**9**) was 73%. Found M^+336 ($C_{25}H_{20}O$ requires M^+ 336.15). δ_H ($CDCl_3$, 300 MHz): 3.37 (2H, dd, $C=CHCH_2$), 3.55 (2H, ab q, J = 13 Hz, CCH_2), 3.54 (2H, d, J = 14 Hz, $PhCH_2$), 6.53 (1H, t, J = 2 Hz, $C=CH$), 7.12 (5H, m, 5 × Ar-H), 7.25 (5H, br m, 5 × Ar-H), 7.47 (2H, m, 2 × Ar-H), 7.78 (1H, d, J = 7 Hz, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 36.8, 37.6, 41.7, 55.5, 120.5, 123.9, 124.2, 124.7, 125.9, 126.1, 126.4, 127.7, 127.2, 130.2, 130.2, 130.4, 134.8, 136.6, 136.7, 143.1, 145.1, 145.1, 152.6.

*7.1.2.7. Methyl 4-((2, 3-dihydro-2-(1H-inden-3-yl)-1-oxo-1H-inden-2-yl)-methyl) benzoate (**10**).* Prepared from (**1**) by an LDA based alkylation. The reaction yield for **10** was 69%. Found M^+376 ($C_{26}H_{16}O_3$ requires M^+ 376.40).). υ_{max} (KBr) 1770.9, 1688.2 cm^{-1} δ_H ($CDCl_3$, 300 MHz): 3.37 (2H, dd J = 1.8 Hz, $C=CHCH_2$), 3.45, 3.54 (2H, d, J = 14 Hz, $PhCH_2$), 3.57 (2H, q J = 13 Hz, C–CH_2), 3.84 (3H, s, CH_3), 6.48 (1H, t, J = 1.8 Hz, $C=CH$), 7.25 (7H, m, 7 × Ar-H), 7.46 (2H, dt, 2 × Ar-H), 7.77 (3H, m, 3 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 36.9, 37.6, 41.6 51.9, 55.4, 128.4, 123.5, 124.0, 124.3, 124.8, 125.9, 126.1, 127.5, 129.2, 130.2, 130.2, 130.6, 135.1, 136.6, 136.7, 142.9, 144.6, 145.1, 152.3, 166.8, 207.2.

*7.1.2.8. Methyl 2-(2, 3-dihydro-2-(1H-inden-3-yl)-1-oxo-1H-inden-2-yl) acetate (**12**).* Prepared from (**1**) by an LDA based alkylation. The reaction yield for **12** was 53%. Found M^+318 ($C_{21}H_{18}O_3$ requires M^+ 318.36).). υ_{max} (KBr) 1768.5, 1690.5 cm^{-1} δ_H ($CDCl_3$, 300 MHz): 3.31 (2H, q J = 16.2 Hz, $COCH_2$), 3.30 (2H, dd, J = 2 Hz, $C=CHCH_2$), 3.54 (3H, s, $COOCH_3$), 3.65 (2H, ab q, CH_2COOCH_3), 6.31 (1H, t, J = 1.8 Hz, CH), 7.25 (3H, m, 3 × Ar-H), 7.42 (3H, m, 3 × Ar-H), 7.63 (1H, dt, 1 × Ar-H), 7.91 (1H, m, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 37.6, 38.2, 39.8, 51.6, 52.0, 120.3, 124.3, 124.8, 125.9, 126.3, 127.5, 130.4, 134.9, 136.1, 142.5, 143.6, 145.0, 152.2, 171.4, 206.2.

*7.1.2.9. 2-(2, 3-Dihydro-2-(1H-inden-3-yl)-1-oxo-1H-inden-2-yl)-acetic acid (**11**).* Benzoate ester (**10**) (0.1 g, 0.253 m mol) was dissolved in a solution of 1.45 M NaOH in 4 ml THF-MeOH-H_2O, (6:3:2). This reaction mixture was refluxed for 20 min. The solution was cooled and saturated NH_4Cl (4 ml), followed by HCl (2 M, 10 mL) was added and the solution was then partitioned with Et_2O. The organic layer was washed and dried to yield 10 which was purified by column chromatography on SiO_2 (n-hexane: EtOAc gradient) to yield **10** as an oil (69%). Found M^+362 ($C_{26}H_{16}O_3$ requires M^+ 362.37).). υ_{max} (KBr) 1769.4 cm^{-1} δ_H ($CDCl_3$, 300 MHz): 3.39, 3.45 (2H, dd J = 1.8 Hz, $C=CHCH_2$), 3.49, 3.57 (2H, d, J = 14 Hz, $PhCH_2$), 3.59 (2H, q J = 13 Hz, C-CH_2), 6.49 (1H, bs, CH), 7.22 (8H, m, 8 × Ar-H), 7.47 (2H, t, 2 × Ar-H), 7.79 (1H, d, 1 × Ar-H), 7.89(2H, d, 2 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 36.9, 37.9, 41.7, 55.4, 120.5, 124.1, 124.3, 124.8, 126.0, 126.2, 127.4, 129.8, 129.8,, 130.2, 130.3, 130.7, 136.4, 135.2, 135.2, 142.9, 143.1, 144.6, 145.1, 152.3, 171.6, 207.3.

7.1.3. General method for reduction with Wilkinson's catalyst

Dimers were dissolved in ethanol and ethyl acetate (1:1) and the resultant solution was stirred at room temperature. To this solution Wilkinson's catalyst was added and the reaction was then stirred under hydrogen for 20 hr. The product was partitioned between ethyl acetate and water and the organic layer was isolated and dried with Na_2SO_4.

*7.1.3.1. 2, 3-Dihydro-2-(1H-inden-3-yl)-2-propylinden-1-one (**5**).* Prepared from **6** using Wilkinson's catalyst. The crude product was purified by flash column chromatography to yield (**5**) 56%. Found M^+286 ($C_{21}H_{18}O$ requires 286.36).δ_H ($CDCl_3$, 300 MHz): 0.88 (3H, t, J = 7 Hz, CH_3), 1.27 (2H, m, CH_2), 2.16 (2H, m, CH_2), 3.36 (2H, br.s, $C=CHCH_2$), 3.49 (2H, ab q, J = 17.6 Hz, $COCH_2$) 6.50 (1H, t, J = 2 Hz, CH), 7.12 (3H, m, 3 × Ar-H), 7.50 (3H, m, 3 × Ar-H), 7.64 (1H, dt, J = 1.2 Hz & J = 7.6 Hz, 1 × Ar-H), 7.86 (1H, d, J = 7.2 Hz, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 14.5 (CH_3), 17.6, 37.6, 38.9, 39.2 (4 × CH_2), 54.3 (qC), 120.3 (CH), 124.1, 124.2, 124.6, 125.8, 126.3, 127.6, 129.8, 135.0, (8 × Ar-CH), 136.9, 143.3, 144.9, 145.3, 152.8 (4 × Ar-C & 1 × C=C), 208.2 (C=O).

*7.1.3.2. 2, 3-Dihydro-2-(1H-inden-3-yl)-2-pentylinden-1-one (**8**).* Prepared from **7** using Wilkinson's catalyst. The crude product was purified by flash column chromatography to yield (**7**) 90%. δ_H ($CDCl_3$, 300 MHz): 0.88 (3H, t, J = 7 Hz, CH_3), 1.27 (2H, m, CH_2), 2.16 (2H, m, CH_2), 3.36 (2H, br.s, $C=CHCH_2$), 3.49 (2H, ab q, J = 17.6 Hz, $COCH_2$) 6.50 (1H, t, J = 2 Hz, CH), 7.12 (3H, m, 3 × Ar-H), 7.50 (3H, m, 3 × Ar-H), 7.64 (1H, dt, J = 1.2 Hz & J = 7.6 Hz, 1 × Ar-H), 7.86 (1H, d, J = 7.2 Hz, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): 14.5 (CH_3), 17.6, 37.6, 38.9, 39.2 (4 × CH_2), 54.3 (qC), 120.3 (CH), 124.1, 124.2, 124.6, 125.8, 126.3, 127.6, 129.8, 135.0, (8 × Ar-CH), 136.9, 143.3, 144.9, 145.3, 152.8 (4 × Ar-C & 1 × C=C), 208.2 (C=O).

7.1.4. Sodium borohydride reduction of dimers

The required dimer was dissolved in ethanol at 0 °C and sodium borohydride was added to the reaction in small portions over 10 min. The reaction was then allowed to reach room temperature and stirred for 3 h. The reaction mixture was poured onto water (20 ml) and extracted into diethyl ether (3 × 20 ml). Flash column chromatography over silica gel afforded the product.

*7.1.4.1. 2, 3-Dihydro-2-(1H-inden-3-yl)-2-propyl-1H-inden-1-ol (**13**).* Prepared by sodium borohydride reduction of (**5**). The reaction yield of (**13**) was 90% and was isolated as a mixture of diastereoisomers (1:2). Where possible the values for the minor diastereoisomer are presented in italics. δ_H ($CDCl_3$, 300 MHz): *0.74 (3H, t, J = 7.4 Hz, $CH_2CH_2CH_3$),*0.84-2.20 (8H, br m, CH_3, CH_2's), 3.29 (2H, s, $C=CHCH_2$), 3.33 (2H, abq, J = 16 Hz, $COHCCH_2$), *3.45 (2H, s, CCH_2), 5.37 (1H, s, CHOH),* 5.42 (1H, br s, CHOH), 6.28 (1H, t, J = 2.1 Hz, C = CH), *6.45 (1H,s,C = CH),* 7.18–7.81 (8H, m, 8 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): *14.4*, 14.8, 18.7 *29.7*, 34.7, 37.5, *39.4*, 40.8, (4 × CH_2), 53.6 (qC), 81.2 (CHOH), 121.7, 124.0, 124.3, 124.4, 124.7, 125.8, 126.7, 128.3, 129.7,*130.9,* 141.7, *142.6*, 144.1, 144.2, 145.3, *146.1,* 148.2, (8 × Ar-CH & 4 × Ar-C & 1 × C=CH & 1 × C = CH).

*7.1.4.2. 2-Allyl-2, 3-dihydro-2-(1H-inden-3-yl)-1H-inden-1-ol (**14**).* Prepared by sodium borohydride reduction of (**6**). The reaction yield for (**14**) was 72% and was isolated as a mixture of diastereoisomers (1:3). Where possible the values for the minor diastereoisomer are presented in italics. δ_H ($CDCl_3$, 300 MHz): *2.24 (1H, d, J = 5.39 Hz, CHOH),* 2.55 & 2.61 (1H, 2 × d, $CCH_2CH=CH_2$), 2.89 &

2.94 (1H, 2 × d J = 6.15 Hz $CC\underline{H}_2CH=CH_2$), 3.33 (2H, g, J = 16 Hz, $C\underline{H}_2$), 3.35 (2H, d, J = 2 Hz, $C=CHC\underline{H}_2$), 4.90 (1H, dd, J = 1 Hz, 10 Hz, $CH_2CH=C\underline{H}_2$), 4.98 (1H, dd, J = 3.3 Hz, 17 Hz, $CH_2CH=C\underline{H}_2$), *5.49 (H, d, J = 5.37 Hz, C<u>H</u>OH),* 5.51 (1H, d, J = 7.9 Hz, C<u>H</u>OH), *5.62 (1H, m, C<u>H</u>=CH$_2$),* 5.64 (1H, m, $C=C\underline{H}CH_2$), 6.40 (1H, s, $C=C\underline{H}$) 7.19 (5H, m, 5 × Ar-<u>H</u>), 7.35 (1H, m, 1 × Ar-H), 7.50 (1H, d, J = 5 Hz, 1 × Ar-H), 7.72 (1H, d, J = 5 Hz, 1 × Ar-H). δ_C ($CDCl_3$, 75.47 MHz): *29.7,* 37.4, 37.6, 40.4, 53.0, *81.7,* 81.8, 116.9, 121.7, 124.1, 124.1, 124.4, 124.7, 125.8, 126.9, 128.4, 129.9, 136.1, 140.7, 143.9, 144.1, 145.3, 148.2.

*7.1.4.3. 2,3-Dihydro-2-(1H-inden-3-yl)-2-((E)-pent-2-enyl)-1H-inden-1-ol (**15**).* Prepared by sodium borohydride reduction of (**7**). The reaction yield for (**15**) was 95% and was isolated as a mixture of diastereoisomers. δ_H ($CDCl_3$, 300 MHz): 0.79 (3H, t, J = 7.4 Hz, $CH_2C\underline{H}_3$), *0.81 (3H, t, J = 7.2 Hz, CH$_2$C<u>H</u>$_3$),* 1.83–3.36 (8H, br m, CH_2's), 5.21–5.53 (2H, m, $CH_2C\underline{H}=C\underline{H}CH_2$), 5.54 (1H, br s, C<u>H</u>OH), *5.68 (1H, bs, C<u>H</u>OH),* 6.21, 6.43 (1H, 2 × s, C<u>H</u>=C), 7.21–7.71 (8H, m, 8 × Ar-<u>H</u>).δ_H (CDC_3, 300 MHz): 0.34–3.52 (12H, m, CH_2's), 5.09–5.29 (2H, m, C<u>H</u>=C<u>H</u>), 5.36 (1H, br.m, C<u>H</u>OH), 6.42 (1H, d, J = 6.5 Hz, C<u>H</u>=C), 7.23–7.76 (8H, m, 8 × Ar-<u>H</u>).

7.2. Pharmacology

7.2.1. Smooth muscle relaxation

Measurement of smooth muscle relaxant activity was performed as previously described [11]. In short, guinea pig (250–400 g, either sex) ileum was removed and 2.5 cm lengths were suspended in a high potassium calcium-free modified Kreb's solution (composition (mM): NaCl 12.5, KCl 45, $MgCl_2$ 1.15, NaH_2PO_4 1.17, $NaHCO_3$ 25, glucose 11.1) at 37 °C, gassed with 95%O_2/5%CO_2 under a resting tension of 1.5 g, from Grass FT.03 transducers fitted with black springs to record contractions isometrically. Contractions were displayed and assessed using MacLab 4 equipment and Chart 3.4 software. Sustained (>40 min) contractures were elicited by addition of $CaCl_2$ solution sufficient to raise the calcium concentration in the bath to 2.5 mM. When contractures had reached a stable maximum, test compounds were administered at a single concentration of 1×10^{-5} M or in the case of sufficiently active compounds, they were added cumulatively (3×10^{-8} M – 3×10^{-5} M).

7.2.2. Mast cell stabilisation

Measurement of mast cell stabilising activity was carried out as decribed previously [13]. Breifly, peritoneal mast cells were harvested from female Wistar rats (250–350 g) in 10 ml of pre-warmed (37 °C) 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) and centrifuged for 6 min at 1000 rpm, using a Sigma 204 centrifuge, and the supernatant removed. The cells were washed 3 times and after the final wash, the pellet was stored at 4 °C for use as soon as practicable. Cells were pretreated with disodium cromoglycate or test compound (2×10^{-5} M) for 10 min before stimulation of histamine release with compound 48/80 (2 μg ml^{-1}). Released histamine was assayed using O-phthaladehyde.

Values are expressed as a mean ± SEM and statistical comparisons between groups was performed by one way Anova, followed by Dunnets Multiple Comparison Test as a post-test. Data were displayed and statistical analysis was performed using Prism 4 software.

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.

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