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Synthesis and pharmacological activity of aminoindanone dimers and related compounds

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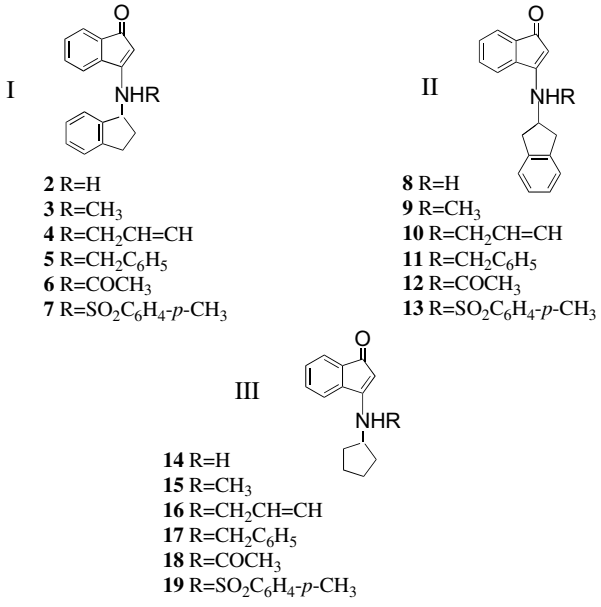
Abstract—A series of N-substituted 3-aminoindanones were synthesised and evaluated for smooth muscle relaxant activity and mediator release inhibition effects. A low level of smooth muscle relaxant activity has been identified in all derivatives. Data have revealed that the significant mediator release inhibition effects observed are related to the nature of the amine substituents. A structure activity relationship is proposed.

1. Introduction

Indane structures occur in many bio-active compounds. The HIV-1 inhibitor Indinavir is one example of a protease inhibitor in clinical use that contains an indane fragment.[1] The nitro-indanone nivemedone was reported to have anti-allergenic activity[2] while Heinzelmann et al. demonstrated the bronchodialatory activity of simple indanols in the 1940s.[3] In addition many synthetic and naturally occurring indanone derivatives have been shown to demonstrate therapeutic effects including smooth muscle relaxant activity[4–6] and mediator release inhibition.[7] As part of our continuing investigation into the pharmacological activity and potential therapeutic activity of naturally occurring and synthetic monomeric[4–6] and dimeric indanes and indanones[7,8] we now report on the smooth muscle relaxant activity and mediator release inhibition activities demonstrated by a series of aminoindanones. In the present work, a series of substituted 1 and 2-*N*-indanyl and cyclopentyl aminoindanones and their derivatives were synthesised and tested for their in vitro smooth muscle relaxant and mediator release inhibition.

2. Chemistry

3-Bromoindanone (**1**) was identified as the key intermediate in the preparation of the starting 3-aminoindanones (Groups **I-III**) used in this study. It was prepared by free radical bromination of indan-1-one using *n*-bromosuccinimide (NBS) and a catalytic amount of dibenzoyl peroxide in CCl_4. Cyclopentyl amine and 1- and 2-aminoindane were subsequently coupled with **1** at room temperature using triethylamine as base and DCM as solvent to yield amines **2**, **8**, **14** (Fig. 1). A range of *N*-alkyl and *N*-acyl derivatives were prepared using standard methods. Compounds were screened as isomeric mixtures for smooth muscle relaxation and mediator release effects.



2 R=H
3 R=CH_3
4 R=$CH_2CH{=}CH$
5 R=$CH_2C_6H_5$
6 R=$COCH_3$
7 R=$SO_2C_6H_4$-*p*-CH_3

8 R=H
9 R=CH_3
10 R=$CH_2CH{=}CH$
11 R=$CH_2C_6H_5$
12 R=$COCH_3$
13 R=$SO_2C_6H_4$-*p*-CH_3

14 R=H
15 R=CH_3
16 R=$CH_2CH{=}CH$
17 R=$CH_2C_6H_5$
18 R=$COCH_3$
19 R=$SO_2C_6H_4$-*p*-CH_3

Keywords: Indanones; 3-Aminoindanones; Bi-indanyl amines; Smooth muscle relaxant activity; Mediator release inhibition.
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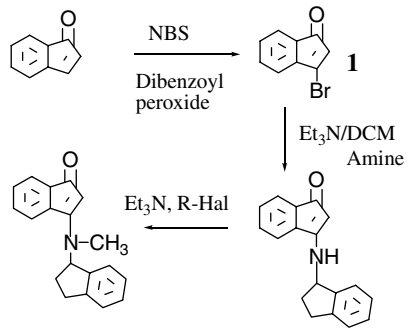

Figure 1.

3. Results and discussion

A total of 18 compounds were screened for their smooth muscle relaxant activity by measuring inhibition of contractions of isolated segments of guinea-pig ileum which were initiated by the addition of 25 μl of 1 M $CaCl_2$ (a final concentration of 2.5 mM). The contractions reached a stable maximum within 5–10 min. and could be maintained for up to 45 min. On stabilisation of the contraction, nifedipine (10^{-8} M) or test compounds (10^{-5} M) were added to the preparation. Nifedipine (10^{-8} M) inhibited calcium contractions by 48.1 ± 2.3% whereas the compounds had only minimal activity (Fig. 2a–c), which was insufficient to allow the construction of a full dose/inhibition curve or the calculation of EC_{50} values. The most potent of the synthetic derivatives was the *N*-methyl, *N*-1-indanyl aminoindan-1-one **2** (Fig. 2a), which inhibited contractions of smooth muscle by 30.0 ± 2.9% at a concentration of 1×10^{-5} M. The *N*-prop-2-enyl and *N*-ethanamide derivatives of the same series (I) **4** and **6** inhibited contractures (20.9 ± 0.8% and 18.3 ± 3.8%, respectively) to a lesser extent. This is in contrast to the findings in our previous studies,[4–6] which showed that synthetic analogues of naturally occurring indanones had EC_{50} values against calcium contractions of ileal smooth muscle ranging from 1.3×10^{-6}M to 1.1×10^{-5} M, with inhibition at 1×10^{-5} M being as high as 70%, for the most active compound. The observed results for the aminoindanone derivatives although not high are in the same order of activity as the naturally occurring indanones onitin (EC_{50} 1×10^{-4} M), onitisin (EC_{50} 2×10^{-3} M) and otinoside (EC_{50} 7×10^{-4} M).[9,10] No obvious structure activity relationship can be determined for this series.

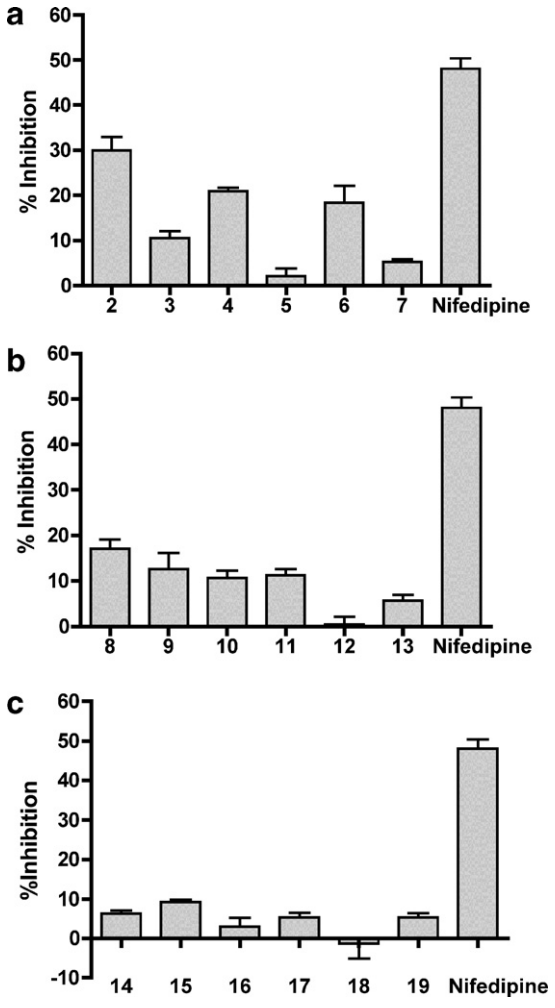

Figure 2.

Mast cells, in addition to their role in allergy, inflammation and anaphylaxis may play a critical role in the induction and maintenance of a variety of severe allergic and auto-immune disease.[11,12] Having previously established that indane dimers inhibit mast cell histamine release[7] the effect of the synthetic aminoindanones was investigated by measuring the inhibition of histamine release from rat peritoneal mast cells stimulated by the addition of compound **48/80**. The positive control disodium cromoglycate (2×10^{-5} M) inhibited mast cell histamine release by 8.1 ± 3.3%. This value is approximately equal to that found by other workers at similar concentrations of cromoglycate.[8] However increasing the concentration of cromoglycate to 1×10^{-4} M failed to give any further increase in inhibition of histamine release (unpublished observations), in contrast to the findings of Tanazaki et al.,[13] who observed an inhibition of histamine release at this concentration of approximately 20%. The magnitude of the cromoglycate response is dependent on both the concentration of compound **48/80** used and also the time of addition of the drug to the cells.[13] Both in this study and that of Walsh et al.,[8] the concentration of Compound **48/80** was high (10 μg/ml), potentially reducing the response of the drug, and in this study, cromoglycate was also added to the cells 10 min prior to compound **48/80**, which may also have had a bearing on the magnitude of the result.

Compound **48/80**-stimulated histamine release was also significantly inhibited by several of the compounds (1×10^{-5} M), ranging from 14.3 ± 2.5% for *N*-acetyl derivative (**6**) to 88.6% ± 0.6 for the *N*-benzyl-derivative **11**. A comparison of the 1-indanylamino-*N*-indanone group (Group I, Fig. 3a) shows that the *N*-prop-2-enyl derivative **4** was the most active compound, inhibiting mast cell histamine release by 79.89 ± 3.67% with the *N*-acetyl derivative **6** being the

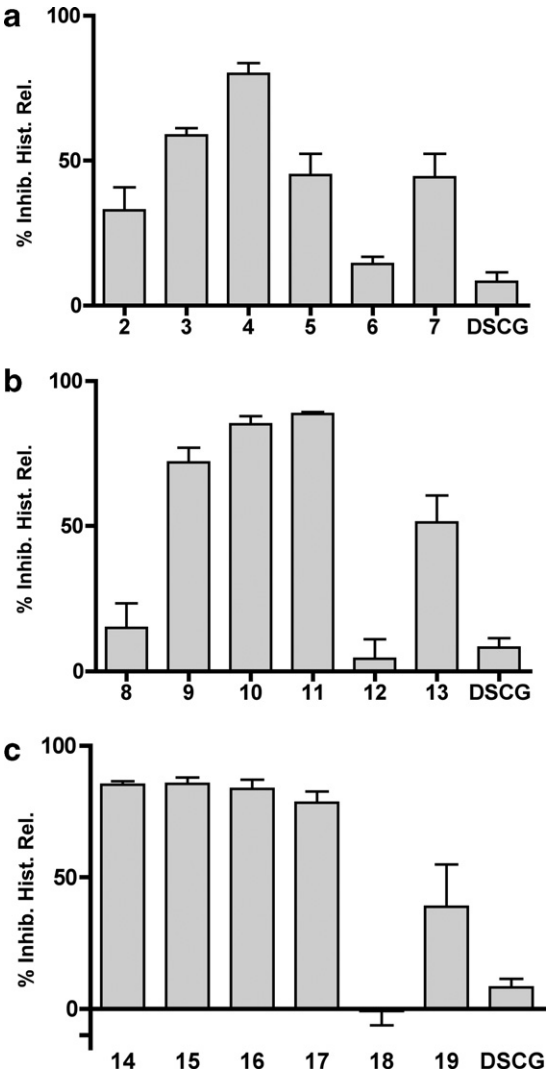

Figure 3.

least potent, inhibiting histamine release by 14.33 ± 2.47%. Compounds in Group II showed activity ranging from 71.88 ± 5.09% for the *N*-benzyl **11** to 4.26 ± 6.8% for the *N*-acetyl derivative **12** (Fig. 3b). There was no significant difference ($P > 0.05$) between the *N*-methyl derivative 9, the *N*-propen-2-yl derivative **10** and the *N*-benzyl derivative **11**. Compounds in Group III showed activity ranging from 85.62 ± 2.36 for the *N*-methyl compound **15** to −0.9 ± 5.4% for the *N*-acetyl derivative **18** (Fig. 3c). There was no significant difference in the activity of **14**, **15**, **16** and **17** ($P > 0.05$). A comparison of similar compounds between the three groups showed that there was no significant ($P > 0.05$) difference in activity between the two *N*-methyl derivatives **3** and **9** and the 3-(*N*-cyclopentyl-*N*-methylamino) indanone (**15**). There was also no significant ($P > 0.05$) difference in activity between the prop-2-enyl derivatives **4**, **10** and **16**. However of the *N*-benzyl derivatives, **11** showed a significant ($P < 0.01$) increase in activity in comparison to **5** whereas there was no significant ($P > 0.05$) difference between **17** and **5**. From these results it appears that the *N*-cyclopentyl aminoindanone derivatives have enhanced activity relative to the 1 and 2 indanyl aminoindanone derivatives with the exception of the *N*-acetyl-cyclopentyl derivative **18** which is the least active of all the derivatives investigated. In general the *N*-acetyl derivatives are the least active of the compounds investigated.

4. Conclusion

We have previously reported[7] that a series of dimeric indanones have mast cell stabilising activity, with the most potent compound investigated in that study, 2-(1-indanyl)-2-methylindane, inhibiting histamine release by 23%. In the current study three compounds, **14**, **15** and **16**, inhibited histamine release by more than 80%. This represents a considerable increase in activity over our earlier studies. These compounds are currently undergoing detailed mechanistic study.

5. Experimental protocols

Melting points were determined on a Me-Opta hot stage and are uncorrected. Infrared spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Ultra violet spectra were recorded on a Varian Carey 3E UV–visible spectrophotometer. Mass spectra were determined at 70 eV on an AEI MS 30 instrument. ^{1}H NMR spectra were recorded on a Bruker MSL 300 instrument at 300 MHz. ^{13}C NMR were recorded at 75.47 MHz. Deuteriochloroform was used as solvent. TLCs were run on commercially pre-coated plates (Merck, Kieselgel $60F_{254}$). Merck Kieselgel 230–400 mesh was used for column chromatography.

5.1. Synthesis of 3-bromoindan-1-one (1)

N-Bromosuccinimide (1 equiv) and dibenzoylperoxide (5 mol%) were added to a solution of indan-1-one (1 equiv) in CCl_4 and the reaction was refluxed for 45 min. After cooling the reaction was washed with water, dried over Na_2SO_4, filtered and evaporated in vacuo. The resultant oil was purified by column chromatography over silica gel (eluant, pet. ether/EtOAc, 4:1) to yield **1** as an oil. δ_H ($CDCl_3$): 3.02 (1H, dd, J = 19 Hz, 2.7 Hz), 3.30 (1H, dd J 6.8, 18.6 Hz), 5.52 (1H, dd, J 6.7, 2.8 Hz, CH), 7.21–7.27 (2H, m, ArH), 7.43 (2H, m, ArH).

5.2. General procedure for the coupling of 3-bromoindan-1-one (1) with 1-aminoindan, 2-aminoindan and cyclopentylamine. Synthesis of 2, 8 and 14

Triethylamine (2 equiv) was added to a stirred solution of 3-bromoindanone (1 equiv) and amine (1 equiv) in dry DCM and the mixture was stirred at 0 °C for 3 h. The solvent was removed in vacuo and the residue was purified directly by flash column chromatography on silica gel (eluant, pet. ether/EtOAc, 4:1).

5.2.1. (*R*,*S*)-3[2,3-Dihydro-1H-inden-1-ylamino]-1H-inden-1-one (2). Prepared by coupling (**1**) with 1-aminoindan.

White solid (60%), mp 117.2–117.9 °C (EtOH). λ_{max} (film) 3035 (ArCH), 1721 (CO), 1616 (NH) cm^{-1}; δ_H ($CDCl_3$): 1.83 (1H, m, CH), 2.47 (1H, m, CH), 2.59 (1H, dd *J* 18.6, 3.5 Hz, CH), 2.85 (1H, m), 3.02 (1H, m), 3.10 (1H, d *J* 6.8, 18.6 Hz, CH), 4.43 (1H, dd, *J* 6.7 Hz, CH), 4.65 (1H, q *J* 3.5, 6.7 Hz, CH), 7.21–7.27 (3H, m, ArH), 7.43 (2H, m, ArH), 7.65 (2H, dt *J* 1.2, 7.6 Hz, ArH), 7.75 (2H, t *J* 7.2 Hz, ArH); δ_C 30.4 (CH_2), 36.0 (CH_2), 46.7 (CH_2), 55.1 (CH), 62.5 (CH), 123.2 (ArCH), 124.0 (ArCH), 124.8 (ArCH), 126.0 (ArCH), 126.3 (ArCH), 127.5 (ArCH), 128.6 (ArCH), 134.8 (ArCH), 136.6 (qC), 143.3 (qC), 145.3 (qC), 156.5 (qC), 204.6 (CO); MS: *m*/*z* 263 [M^+].

5.2.2. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-ylamino]-1H-inden-1-one (8). Prepared by coupling **1** with 2-aminoindan. Buff solid (71%), mp 103–104 °C (EtOH). λ_{max} (KBr) 3051 (ArCH), 1706 (CO), 1604 (NH) cm^{-1} δ_H ($CDCl_3$): 2.58 (1H, dd *J* 3.4, 18.5 Hz, CH), 2.84 (1H, dd *J* 6.8, 4.5 Hz, CH), 2.86 (1H, dd *J* 6.9, 14.1 Hz, CH), 2.99 (1H, dd *J* 6.7, 18.5 Hz, CH), 3.17 (1H, dd *J* 6.9, 19.1 Hz, CH), 3.23 (1H, dd *J* 6.9, 19.3 Hz, CH), 3.81 (1H, quin *J* 7.0 Hz, CH), 4.51 (1H, q*J* 3.1, 6.7 Hz, CH), 7.21 (4H, m, ArH), 7.43 (1H, m, ArH), 7.67 (3H, m, ArH), δ_C 39.9 (CH_2), 40.6 (CH_2), 45.4 (CH_2), 54.5 (CH), 58.1 (CH), 123.1 (ArCH), 124.4 (ArCH), 124.4 (ArCH), 124.4 (ArCH), 125.7 (ArCH), 125.7 (ArCH), 128.4 (ArCH), 134.6 (ArCH), 136.4 (qC), 141.1 (qC), 141.2 (qC), 155.9 (qC), 204.2 (CO); MS: *m*/*z* 263 [M^+].

5.2.3. (*R*,*S*)-3(*N*-*R*,*S*)-1Cyclopentylamino)-indan-1-one (14). Prepared by coupling **1** with cyclopentylamine. Oil (85.8%), λ_{max} (film) 3022 (ArCH), 1743 (CO), 1426 (CH) cm^{-1}; δ_H ($CDCl_3$): 1.65 (9H, bm), 2.50 (1H, dd *J* 3.1, 18.7 Hz), 3.01 (1H, dd *J* 6.6, 18.7 Hz), 3.25 (1H, quin *J* 3.2 Hz), 4.45 (1H, q *J* 7.2, 18.7 Hz), 7.70 (1H, m, ArH), 7.45 (3H, m, ArH), δ_C 23.7 (CH_2), 23.8 (CH_2), 33.1 (CH_2), 34.0 (CH_2), 45.6 (CH_2), 54.8 (CH), 58.3 (CH), 123.2 (ArCH), 125.9 (ArCH), 128.5 (ArCH), 134.7 (ArCH), 136.6 (qC), 156.4 (qC), 204.8 (CO); MS *m*/*z*: 214 [M^+].

5.3. General procedure for the alkylation of 2, 8 and 14. Synthesis of 3–5, 9–11 and 15–17

Triethylamine (1.2 equiv) and alkyl halide (5 equiv) were added to a solution of amine (1 equiv) in DCM and the reaction mixture was stirred at room temperature for 2 h, the solvent was evaporated in vacuo and the residue directly purified by column chromatography over silica gel (pet. Ether/EtOAc, 4:1) to yield.

5.3.1. (*R*,*S*)-3[2,3-Dihydro-1H-inden-1-yl(methyl)amino]-1H-inden-1-one(3). Prepared by methylation of **2** with MeI. Oil (45%), λ_{max} (film) 3063 (ArCH), 1730 (CO), 1604 (NH) cm^{-1}; δ_H ($CDCl_3$): 1.89 (3H, s, CH_3), 2.08 (1H, m, CH), 2.62 (1H, dd *J* 3.7, 6.9 Hz, CH), 2.81 (1H, m, CH), 2.97 (1H, m, CH), 4.34 (1H, t *J* 7.7 Hz, CH), 4.55 (1H, dd *J* 3.7, 6.9 Hz, CH), 4.63 (1H, t *J* 7.7 Hz), 4.77 (1H, dd *J* 3.7, 6.9 Hz), 7.24 (3H, m, ArH), 7.44 (2H, m, ArH), 7.65 (1H, dq *J* 1.4, 7.3 Hz, ArH), 7.75 (1H, dt *J* 7.7 Hz, ArH), 7.84 (1H, d *J* 7.7 Hz, CH); δ_C 26.6, 27.2 (CH_2), 28.0 (CH), 30.3, 30.5 (CH_2), 34.3 (CH_3), 38.0, 38.9 (CH_2), 58.1 (CH), 61.8 (ArCH), 67.0 (ArCH), 70.0 (ArCH), 122.8 (ArCH), 124.5, 124.8 (ArCH), 126.2, 126.4 (ArCH), 127.4 (ArCH), 128.4 (ArCH), 134.7, 134.8 (qC), 142.9 (qC), 143.7 (qC), 156.0 (qC), 204.6 (CO); MS: *m*/*z* 277 [M^+].

5.3.2. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-yl(methyl)amino]-1H-inden-1-one (9). Prepared by methylation of **8** with MeI. White solid (40%), mp 120.4–120.9 °C, λ_{max} (KBr) 3075, 2985, 1707, 1602 cm^{-1}; δ_H ($CDCl_3$): 2.03 (3H, s, NCH_3), 2.57 (1H, dd *J* 7.0, 18.9 Hz, CH), 2.77 (1H, dd *J* 3.8, 18.9 Hz, CH), 3.05 (4H, m), 3.52 (1H, quin *J* 8 Hz, CH), 4.78 (1H, q *J* 3.8, 6.8 Hz, CH), 7.20 (4H, m, ArH), 7.43 (1H, dd *J* 6.8, 7.5 Hz, ArH), 7.65 (1H, dd *J* 6.1, 7.5 Hz, ArH), 7.75 (2H, t, *J* 6.1, 6.8 Hz, ArH); δ_C 33.1 (CH_3), 35.8 (CH_2), 37.7 (CH_2), 38.0 (CH_2), 59.6 (CH), 65.1 (CH), 123.0 (ArCH), 124.3 (ArCH), 124.3 (ArCH), 126.4 (ArCH), 126.4 (ArCH), 128.4 (ArCH), 129.0 (ArCH), 134.7 (ArCH), 137.2 (qC), 141.2 (qC), 141.4 (qC), 155.6 (qC), 205.0 (CO); MS: *m*/*z* 277 [M^+].

5.3.3. (*R*,*S*)-3(*N*-Cyclopentyl-*N*-methylamino)-indan-1-one (15). Prepared by methylation of **14** with MeI. Oil (80%), λ_{max} (Film) 3054, 2989, 1707, 1609 cm^{-1}; δ_H ($CDCl_3$): 1.66 (8H, m), 1.94 (3H, s, NCH_3), 2.62 (1H, q *J* 18.9 Hz, CH), 2.66 (1H, q *J* 19.1 Hz, CH), 2.84 (1H, m *J* 8.1 Hz, CH), 4.79 (1H, t *J* 3.2 Hz, CH), 7.41 (1H, t *J* 7.2 Hz, ArH), 7.63 (1H, t *J* 7.2 Hz, ArH), 7.72 (2H, m, ArCH); δ_C 23.8 (CH_2), 23.8 (CH_2), 31.4 (CH_2), 31.4 (CH_2), 35.4 (CH_2), 33.5 (CH_3), 59.9 (CH), 65.4 (CH), 123.0 (ArCH), 126.6 (ArCH), 128.4 (ArCH), 132.4 (ArCH), 137.3 (qC), 152.3 (qC), 204.3 (CO); MS: *m*/*z* 229 [M^+].

5.3.4. (*R*,*S*)-3[2,3-Dihydro-1H-inden-1-yl(prop-2-enyl)-amino]-1H-inden-1-one (4). Prepared by alkylation of **2** with allyl bromide. Oil (35%), λ_{max} (film) 3087 (ArCH), 1722 (CO), 1599 (NH) cm^{-1}; δ_H ($CDCl_3$): 2.05 (2H, bm, CH_2), 2.47 (1H, dd *J* 9.5 Hz, CH), 2.72 (2H, m, CH_2), 3.11 (2H, bm, CH), 4.40 (1H, t *J* 3.0 Hz, CH), 4.50 (1H, t *J* 3.0 Hz, CH), 4.65 (1H, m, CH), 5.15 (2H, bm, *CH*=CH_2), 5.80 (1H, bm, CH=*CH*$_2$), 7.20 (3H, bm, ArH), 7.40 (1H, m, ArH), 7.50 (1H, m, ArH), 7.64 (1H, bm, ArH), 7.74 (1H, m, ArH), 7.86 (1H, m, ArH); δ_C 27.9 (CH_2), 30.2 (CH_2), 41.3 (CH_2), 49.4 (CH_2), 57.0 (CH), 64.6 (CH), 116.4 (=CH_2), 122.9 (ArCH), 124.1 (ArCH), 124.7 (ArCH), 126.1 (ArCH), 126.4 (ArCH), 127.3 (ArCH), 128.4 (ArCH), 134.7 (CH), 137.1 (qC), 137.2 (ArCH), 143.2 (qC), 144.4 (qC), 156.7 (qC), 204.9 (CO); MS: *m*/*z* 303 [M^+].

5.3.5. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-yl(prop-2-enyl)-amino]-1H-inden-1-one (10). Prepared by alkylation of **8** with allyl bromide. Oil (40.8%). λ_{max} (film) 3062, 2985, 1722, 1603 cm^{-1}; δ_H ($CDCl_3$): 2.65 (1H, dd *J* 6.7, 18.8 Hz, CH), 2.68 (1H, dd *J* 4.2, 18.8 Hz, CH), 2.98 (6H, m), 3.77 (1H, quin *J* 7.6 Hz, CH), 4.67 (1H, dd *J* 4.2, 6.7 Hz, CH), 5.05 (1H, dd *J* 1.8, 10.2 Hz, CH), 5.08 (2H, dd *J* 1.8, 10.2 Hz, CH=CH_2), 5.80 (1H, m, =CH), 7.15 (4H, m, ArH), 7.42 (1H, dt *J* 7.8 Hz, ArH), 7.64 (1H, dt *J* 1.2, 7.7 Hz, ArH), 7.74 (2H, dd *J* 1.2, 7.0 Hz); δ_C 35.9 (CH_2), 38.6 (CH_2), 39.1 (CH_2), 50.1 (CH_2), 57.3 (CH), 61.0 (CH), 116.3 (CH_2),

122.9 (ArCH), 124.1 (ArCH), 124.5 (ArCH), 126.3 (ArCH), 126.3 (ArCH), 126.4 (ArCH), 128.4 (ArCH), 134.7 (CH), 137.2 (ArCH), 141.3 (qC), 141.5 (qC), 156.3 (qC), 156.3 (qC), 204.7 (CO); MS: *m/z* 303 [M^+].

5.3.6. (*R*,*S*)-3(*N*-prop-2-enyl-*N*-2-indanylamino)-indan-1-one (10). Prepared by alkylation of **14** with allyl bromide. Oil (82.6%). λ_{max} (film) 3056 (ArH), 1728 (CO), 1602 (NH) cm^{-1}; δ_H ($CDCl_3$): 1.65 (6H, m), 2.67 (2H, dq *J* 4.4, 19.0 Hz, CH_2), 3.01 (3H, m), 4.71 (1H, dd *J* 6.2, 4.4 Hz, CH), 4.95 (2H, dd *J* 1.3, 10.2 Hz), 5.09 (2H, dd *J* 1.3, 17.3 Hz, =CH), 5.76 (1H, m, =CH), 7.40 (1H, t, ArH), 7.66 (3H, m, ArH); δ_C 23.2 (CH_2), 23.7 (CH_2), 29.7 (CH_2), 31.4 (CH_2), 39.0 (CH_2), 50.8 (CH), 57.9 (CH), 115.6 (=CH_2), 122.8 (=CH), 126.5 (ArCH), 128.2 (ArCH), 134.5 (ArCH), 137.9 (ArCH), 137.9 (qC) 156.6 (qC), 204.9 (CO); MS: *m/z* 255 [M^+].

5.3.7. (*R*,*S*) 3[2,3-Dihydro-1H-inden-1-yl(benzyl)amino]-1H-inden-1-one (5). Prepared by reaction of **2** with benzyl bromide. Oil (85%), λ_{max} (film) 3094 (ArH), 1714 (CO), 1595 (NH) cm^{-1}; δ_H ($CDCl_3$): 2.65 (1H, dq *J* 7.1, 19.3 Hz, CH), 2.76 (1H, dq *J* 3.7, 19.3 Hz, CH), 2.52 (4H, m), 3.60 (1H, q *J* 12.8, 17.9 Hz, CH), 3.75 (1H, q *J* 12.8, 17.9 Hz, CH), 4.39 (1H, dt *J* 7.3, 8.2 Hz, CH), 4.61 (1H, dd, *J* 4.0, 7.0 Hz, CH), 7.45 (11H, m, ArH), 7.85 (1H, d *J* 7.2 Hz, ArH), 8.01 (1H, d *J* 7.2 Hz, ArH), δ_C 39.5 (CH_2), 40.1 (CH_2), 50.6 (CH_2), 55.6 (CH_2), 56.2 (CH), 63.2 (CH), 122.8 (ArCH), 124.0 (ArCH), 124.5 (ArCH), 126.2 (ArCH), 126.3 (ArCH), 126.3 (ArCH), 126.8 (ArCH), 128.0 (ArCH), 128.2 (ArCH), 128.4 (ArCH), 134.7 (ArCH), 139.6 (ArCH), 143.1 (ArCH), 143.4 (qC), 143.6 (qC), 144.2 (qC), 156.2 (qC), 156.5 (qC), 204.8 (CO); MS: *m/z* 353 [M^+].

5.3.8. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-yl(benzyl)amino]-1H-inden-1-one (11). Prepared by benzylation of **8** with benzyl bromide. Oil (40%), λ_{max} (film) 3093 (ArH), 1728 (CO), 1693 (CH) cm^{-1}; δ_H ($CDCl_3$): 2.63 (1H, dd *J* 6.7, 18.8 Hz, CH), 2.81 (1H, dd *J* 3.8, 18.8 Hz, CH), 3.04 (4H, m), 3.64 (2H, m, CH_2), 3.76 (1H, t *J* 6.7 Hz), 4.67 (1H, m, CH), 7.31 (10H, m, ArH), 7.67 (1H, dt *J* 1.2, 7.7 Hz, ArH), 7.76 (1H, dt *J* 1.2, 7.7 Hz, ArH), 7.87 (1H, t *J* 7.7 Hz, ArH); δ_C 35.2 (CH_2), 38.6 (CH_2), 38.7 (CH_2), 50.9 (CH_2), 56.9 (CH), 60.4 (CH), 122.8 (ArCH), 124.0 (ArCH), 124.5 (ArCH), 126.2 (ArCH), 126.3 (ArCH), 126.3 (ArCH), 126.8 (ArCH), 128.0 (ArCH), 128.0 (ArCH), 128.2 (ArCH), 128.2 (ArCH), 128.4 (ArCH), 134.7 (ArCH), 137.1 (qC), 139.9 (qC), 141.2 (qC), 141.4 (qC), 156.1 (qC), 204.6 (CO); MS: *m/z* 353 [M^+].

5.3.9. (*R*,*S*)-3(*N*-Cyclopentyl-*N*-benzylamino)-indan-1-one (17). Prepared by benzylation of **14** with benzyl bromide. Oil (65%), λ_{max} (film) 3022, 2986, 1743, 1421 cm^{-1}; δ_H ($CDCl_3$): 1.51 (8H, br m), 2.75 (2H, d *J* 5.5, CH_2), 3.05 (1H, t *J* 7.7 Hz, CH), 3.56 (2H, dd *J* 3.5, 14.5 Hz, CH_2), 4.68 (1H, t *J* 5.5 Hz, CH) 7.29 (6H, br m, ArH), 7.60 (1H, t *J* 7.3 Hz, ArH), 7.71 (1H, m, ArH), 7.76 (1H, m, ArH); δ_C 23.4 (CH_2), 31.3 (CH_2), 32.1 (CH_2), 42.7 (CH_2), 42.8 (CH_2), 54.2 (CH_2), 55.8 (CH), 57.6 (CH), 122.9 (ArCH), 126.5 (ArCH), 126.8 (ArCH), 127.9 (ArCH), 128.1 (ArCH), 128.1 (ArCH), 128.1 (ArCH), 128.4 (ArCH), 134.8 (ArCH), 137.3 (qC), 140.9 (qC), 151.7 (qC), 156.7 (qC), 205.1 (CO); MS: *m/z* 305 [M^+].

5.4. General procedure for the acylation of 2, 8 and 14. Synthesis of 6, 12 and 18

Triethylamine (2.0 equiv) acetic anhydride (2 equiv) and DMAP (1 equiv) were added to a solution of amine (1.0 equiv) in DCM and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was washed with H_2O, dried over Na_2SO_4, filtered and evaporated in vacuo to yield a residue that was purified by column chromatography over silica gel (pet. ether/EtOAc, 4:1) to yield.

5.4.1. (*R*,*S*)-3[2,3-Dihydro-1H-inden-1-yl(ethanamide) amino)1H-inden-1-one (6). Prepared by acetylation of **2**. Pale solid mp 134.0–134.2 °C (62.5%), λ_{max} (KBr) 3056, 2991, 1720, 1644 cm^{-1}; δ_H ($CDCl_3$): 2.14 (3H, s CH_3), 2.62 (6H, br m), 4.20 (1H, m, CH), 5.51 (1H, m, CH), 7.45 (8H, br m, ArH); δ_C 27.4 (CH_3), 29.8 (CH_2), 29.8 (CH_2), 30.1 (CH_2), 42.8 (CH), 52.4 (CH), 123.3 (ArCH), 124.2 (ArCH), 124.5 (ArCH), 125.4 (ArCH), 125.5 (ArCH), 127.1 (ArCH), 127.2 (ArCH), 127.8 (ArCH), 134.4 (qC), 138.2 (qC), 143.4 (qC), 152.5 (qC), 202.2 (CO), 203.1 (CO);MS: *m/z* 305 [M^+].

5.4.2. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-yl(ethanamide) amino)1H-inden-1-one (12). Prepared by acetylation of **8**. White solid mp 151.1–151.6 °C (55%), λ_{max} (KBr) 3049 (ArH), 1724 (CO), 1641 (CH) cm^{-1}; δ_H ($CDCl_3$: 2.15 (3H, s, CH_3), 2.97 (8H, bm), 7.47 (8H, bm, ArH); δ_C 20.9 (CH_3), 23.0 (CH_2), 23.8 (CH_2), 29.5 (CH_2), 52.3 (CH), 55.9 (CH), 123.3 (ArCH), 124.4 (ArCH), 124.5 (ArCH), 126.0 (ArCH), 127.2 (ArCH), 129.5 (ArCH), 134.5 (ArCH), 135.6 (ArCH), 137.5 (qC), 139.6 (qC), 141.0 (qC), 154.0 (qC), 201.5 (CO), 202.8 (CO); MS: *m/z* 305 [M^+].

5.4.3. *N*-Cyclopentyl-*N*-(*R*,*S*)-3-indan-1-onylethanamide (18). Prepared by acetylation of **14**. Cream solid mp 164.2–164.9 °C (54%), λ_{max} (KBr) 3062 (ArH), 1715 (CO), 1631 (CH) cm^{-1}; δ_H ($CDCl_3$: 2.01 (10H, bm), 2.10 (2H, bm, CH_2), 2.89 (1H, bm, CH), 4.14 (1H, bm, CH), 4.76 (1H, bm, CH), 7.42 (1H, bm, ArH), 7.58 (2H, bm, ArH), 7.82 (1H, bm, ArH); δ_C 23.0 (CH_3), 24.0 (CH_2), 25.4 (CH_2), 29.4 (CH_2), 30.9 (CH_2), 42.2 (CH_2), 52.0 (CH), 56.9 (CH), 123.4 (ArCH), 125.5 (ArCH), 127.8 (ArCH), 129.3 (ArCH), 134.5 (qC), 135.3 (qC), 169.1 (CO), 204.(CO): MS *m/z* 257 [M^+].

5.5. General procedure for the preparation of *p*-toluene sulfonamide derivatives of 2, 8 and 14. Synthesis of 7, 13 and 19

p-Toluene sulfonyl chloride (10 equiv) and pyridine (excess) were added to a stirring solution of amine (1 equiv) in DCM and the reaction mixture was stirred for 0.5 h. at 0 °C and then at room temperature for a further 12 h. After this time the reaction was extracted into aq. HCl (2 M) and ether (1:1, v:v) and the organic layer was

washed (H_2O, ×3), dried over Na_2SO_4, filtered and the solvent evaporated in vacuo. The resulting residue was directly purified by column chromatography over silica gel (pet. ether/EtOAc, 3:1) to yield.

5.5.1. (*R*,*S*)-3[2,3-Dihydro-1H-inden-1-yl(*p*-toluene sulfonamide)amino]-1H-inden-1-one (7). Prepared by sulfonylation of **2**. White needles mp 212.4–212.9 °C (43%), λ_{max} (KBr) 3050, 2989, 1721, 1609 cm^{-1}; δ_H ($CDCl_3$): 2.44 (3H, s CH_3), 2.53 (6H, br m), 4.82 (1H, m, CH), 5.33 (1H, m, CH), 7.43 (12H, br m, ArH); δ_C 21.5 (CH_3), 30.3 (CH_2), 31.1 (CH_2), 44.5 (CH_2), 54.1 (CH), 63.8 (CH), 123.4 (ArCH), 123.8 (ArCH), 124.0 (ArCH), 124.3 (ArCH), 124.5 (ArCH), 125.0 (ArCH), 125.5 (ArCH), 125.8 (ArCH), 127.2 (ArCH), 128.6 (ArCH), 129.5 (ArCH), 134.6 (ArCH), 137.1 (qC), 138.7 (qC), 140.7 (qC), 143.5 (qC), 143.8 (qC), 152.5 (qC), 202.6 (CO); MS: *m*/*z* 417 [M^+].

5.5.2. (*R*,*S*)-3[2,3-Dihydro-1H-inden-2-yl(*p*-toluene sulfonamide)amino]-1H-inden-1-one (13). Prepared by sulfonylation of **8**. Oil (90%), λ_{max} (KBr) 3098 (ArH), 1721 (CO), 1608 (CH) cm^{-1}; δ_H ($CDCl_3$): 2.39 (4H, bs, 2 × CH_2), 2.48 (3H, s, CH_3), 3.00 (2H, br m, CH_2), 3.52 (2H, br m, 2×CH), 7.14–7.50 (12H, br m, ArH); δ_C 21.5 (CH_3), 37.4 (CH_2), 37.0 (CH_2), 38.0 (CH_2), 54.8 (CH), 57.9 (CH), 123.1 (ArCH), 124.0 (ArCH), 124.2 (ArCH), 125.0 (ArCH), 125.6 (ArCH), 126.9 (ArCH), 126.9 (ArCH), 127.9 (ArCH), 128.7 (ArCH), 128.9 (ArCH), 129.5 (ArCH), 134.6 (ArCH), 136.9 (qC), 137.5 (qC), 138.2 (qC), 139.7 (qC), 143.3 (qC), 151.9 (qC), 201.8 (CO); MS: *m*/*z* 417 [M^+].

5.5.3. *N*-Cyclopentyl-*N*-(*R*,*S*)-3-indan-1-onyl-*p*-toluene sulfonamide (19). Prepared by sulfonylation of **14**. Waxy oil (45%), λ_{max} (KBr) 3094 (ArH), 1708 (CO), 1596 (NH) cm^{-1}; δ_H ($CDCl_3$): 2.10 (4H, br m), 2.50 (3H, s, CH_3), 3.50 (2H, br m, CH_2), 3.23 (2H, br m, CH_2), 3.00 (2H, br m, CH_2), 3.52 (2H, br m, 2 × CH), 7.55 (8H, br m, ArH); δ_C 21.4 (CH_3), 23.3 (CH_2), 23.3 (CH_2), 23.7 (CH_2), 30.8 (CH_2), 31.9 (CH_2), 54.7 (CH), 59.1 (CH), 123.3 (ArCH), 125.9 (ArCH), 127.0 (ArCH), 129.1 (ArCH), 129.1 (ArCH), 129.6 (ArCH), 134.9 (ArCH), 134.9 (ArCH), 137.1 (qC), 138.6 (qC), 143.2 (qC), 152.5 (qC), 202.5 (CO); MS: *m*/*z* 341 [M^+].

5.6. Pharmacological methods

5.6.1. Inhibition of smooth muscle contraction. Smooth muscle relaxant activity was assessed as inhibition of calcium contractures of potassium-depolarised guinea-pig ileum as described previously.[4] Guinea pigs (250–400 g) of either sex were killed by cervical dislocation and exsanguination. The abdomen was opened by midline incision and the ileum removed. The tissue was stored at 4 °C in Kreb's solution (composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.15, NaH_2PO_4 1.17, $NaHCO_3$ 25, glucose 14.4). Segments of ileum 2.5 cm in length 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. Isometric contractions were recorded using a MacLab/4e system in conjunction with the Chart 3.3.1 software package.

Sustained (>40 min) contractures were elicited by addition of CaCl solution sufficient to raise the calcium concentration in the bath to 2.5 mM. Test compounds (1×10^{-5} M) and nifedipine (1×10^{-8} M), as a positive control, were prepared in 0.5% (v/v) dimethylsulphoxide (DMSO) and distilled water. The test compound solution was added to the organ bath once a stable contraction of the tissue had been achieved.

5.6.2. Inhibition of compound 48/80-induced histamine release from mast cells. The effect of compounds on Compound **48/80**-induced histamine release from rat peritoneal mast cells was assessed as described previously.[7] Briefly, Female Wistar rats (250–350 g) were killed in an atmosphere of saturated CO_2. A volume of 10 ml of prewarmed (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 mM; Glucose 5.6 mM; HEPES 10 mM) was injected ip and the abdomen was massaged for 3 min. The BSS, along with suspended mast cells and other cells, were aspirated, using a 10 ml syringe, following a mid-line incision. The aspirate was centrifuged for 6 min at 1000 rpm, using a Sigma 204 centrifuge, and the supernatant removed. The cells were washed three times by re-suspending in BSS, at 4 °C, and re-centrifuging. Following the final wash, the pelleted cells were stored at 4 °C, for use as soon as possible. The harvesting of mast cells and the release of histamine from these mast cells was carried out according to a modified protocol described by both Loeffler et al.[14] and Amellal et al.[15] The mast cells were not purified by density gradient centrifugation in accordance with Loeffler et al.[14] where it was demonstrated that consistent and uniform responses to compound **48/80** and other releasing agents were achieved without cell purification.

Test compounds (2×10^{-5} M) and disodium cromoglycate (2×10^{-5} M) were prepared in 0.5% DMSO (0.5% v/v) in distilled water. Histamine release was stimulated by Compound **48/80** (10 μg/ml) following 10 min incubation with test compound or DSCG. Cell stimulation was stopped after 2 min by the addition of 0.5 ml BSS (4 °C) and the incubation tubes were transferred immediately to an ice bath. Basal histamine release was measured in the absence of Compound **48/80**, and total histamine content was assayed following heating to 100 °C for 2 min.

The histamine assay was carried out according to a modified protocol described by Shore et al.[16] A volume of 0.4 ml of 1 M NaOH and 0.1 ml *O*-phthaldialdehyde (oPT) (1% (w/v) in methanol) was added to 2 ml of supernatant from each tube. This was incubated at room temperature for 4 min. The reaction was stopped by the addition of 0.2 ml of 3 M HCl. The presence of the fluorescent product of the reaction was measured using a Shimadzu RF-1501 spectrophotometer set at λ_{ex} = 360 nm and λ_{em} = 450 nm. The supernatant from each incubation tube was assayed in duplicate.

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.

5.7. Statistics

Results are expressed as means ± SEM. Statistical analysis was performed using one way ANOVA, followed by Bonferroni's Multiple Comparison Test. Probability value (P) of less than 0.05 was taken as significant. n denotes the number of animals from which preparations were used in that series of experiments.

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