Bioorganic & Medicinal Chemistry Letters 19 (2009) 5927–5930

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Diastereoisomers of 2-benzyl-2, 3-dihydro-2-(1*H*-inden-2-yl)-1*H*-inden-1-ol: Potential anti-inflammatory agents

Helen Sheridan *, John J. Walsh, Carina Cogan, Michael Jordan, Tom McCabe, Egle Passante, Neil H. Frankish

Trinity College Dublin, School of Pharmacy and Pharmaceutical Sciences, Dublin 2, Ireland

ARTICLE INFO

Article history:
Received 2 July 2009
Revised 12 August 2009
Accepted 13 August 2009
Available online 21 August 2009

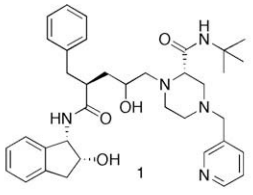
Keywords:
Indane
Indane dimers
Mast cells
Anti-inflammatory
RBL-2H3 cells

ABSTRACT

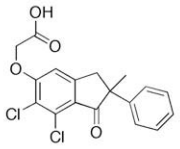
The synthesis and biological activity of the novel diastereoisomers of 2-benzyl-2,3-dihydro-2-(1*H*-inden-2-yl)-1*H*-inden-1-ol is reported. The 2,2-coupled indane dimers were synthesised by coupling of the silyl enol ether of 1-indanone with the dimethyl ketal of 2-indanone. The coupled product was directly alkylated to give the racemic ketone which was reduced to the diastereoisomeric alcohols. The alcohols were separated and their relative stereochemistry was established by X-ray crystallography. These molecules demonstrate significant anti-inflammatory activity in vivo and in vitro and may represent a new class of anti-inflammatory agent.

Introduction. Mast cells contain or may produce a wide range of pro-inflammatory mediators and will degranulate following both immunogenic (IgE) and non-immunogenic stimuli such as superoxides, complement, neuropeptides, and lipoproteins. In addition to histamine, mast cells may also release leukotrienes, and prostanoids, many inflammatory cytokines and chemokines as well as the protease enzymes, tryptase and chymase and many of these mediators are key agents in inflammation.[1] As a consequence of their 'sentinal' locations and their panoply of pro-inflammatory mediators, mast cells may play an active role in many inflammatory diseases, such as allergy, asthma, arthritis, atherosclerosis, pulmonary fibrosis, and parasitic diseases. Mast cells may also play a vital role in host defense against pathogens as part of the innate immune system since they express Toll-like receptors (TLR), and respond to TLR ligands such as LPS and peptidoglycan.[2,3]

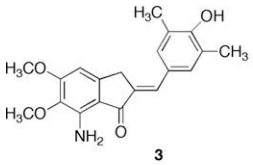
The indane skeleton features in a range of molecules that display significant and diverse biological activity. The protease inhibitor Indinavir **1** (Crixivan) is used as a component of Highly Active Retroviral Therapy (HART) to treat HIV infection and AIDS.[4] Indacrinone **2** is a potent antidiuretic[5] while indanocine **3** demonstrates significant binding with microtubules.[6] For some time this research group has been working on a group of small biologically active indanes and their dimers. We have established that a number of simple indanes related to the pterosin group of natural products.[7–9] and their 1,2-coupled dimers[10,11] demonstrate smooth muscle relaxant activity and also inhibit histamine release from mast cells. The 1,2-coupled indane dimer **4** has shown significant inhibition of compound 48/80-stimulated histamine release from rat peritoneal mast cells together with moderate smooth muscle relaxation effects which suggested that this class of molecule had potential in the treatment of asthma and warranted further investigation.[11] More recently we have identified a second 1,2-coupled indane dimer **5** with significantly increased mast cell stabilisation activity. However, we did not succeed in our initial aim of combining bronchodilatory and mast cell stabilisation activity in a single molecule.[12]



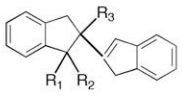
1



2



3



4 R1 = H, R2 = H, R3 = CH_3
5 R1 = H, R2 = OH, R3 = $CH_2CH{:}CHCH_2CH_3$

We have subsequently synthesized and evaluated the activity of related 2,2-coupled indane dimers. We now report on a pair of diastereoisomers (**6** and **7**) which demonstrate significant mast cell

* Corresponding author. Tel.: +353 1 8962825/8.
E-mail addresses: hsheridn@tcd.ie (H. Sheridan), nfrnkish@tcd.ie (N.H. Frankish).

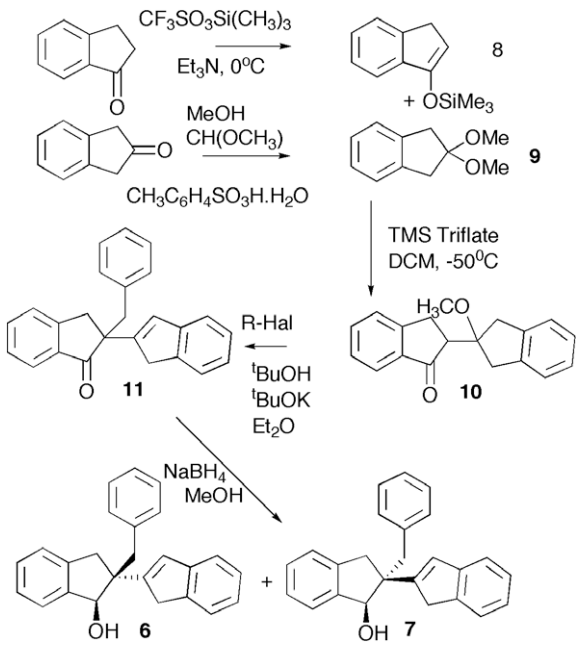
0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.08.060

stabilisation and anti-inflammatory effects in a range of in vivo and in vitro screens. We feel that these diastereoisomers represents a potential new class of indane compounds with potential to treat a range of inflammatory conditions. The target 2,2-coupled indane dimers were synthesised as shown in Scheme 1.[13] The key intermediate to this class of dimer **11**, was prepared from **10** which was synthesised by coupling of the silyl enol ether of 1-indanone **8** with the dimethyl ketal of 2-indanone **9**. The coupled product **10** was alkylated to yield **11**. This route has been successfully used to synthesise a range of related dimers. Reduction of **11** yielded the diastereoisomers **6** and **7**. The relative stereochemistry of the pair of diastereoisomers was established by single crystal X-ray analysis of **6** as shown in Figure 1.[14]

Compounds **6** and 7 were evaluated for their smooth muscle relaxant activity, their ability to inhibit histamine release from rat peritoneal mast cells and to inhibit calcium ionophore A23187-stimulated β-hexosaminidase release from RBL-2H3 cells; these latter cells are a histamine-releasing cell line commonly used to model mast cell degranulation.[15] β-Hexosaminidase is released in parallel with histamine and both histamine and β-hexosaminidase release from rat peritoneal mast cells and RBL-2H3 cells, respectively, were used as markers for inhibition of release of inflammatory mediators from the mast cell. The compounds were also assessed for broad anti-inflammatory activity in vivo using the arachidonic mouse ear swelling test.

Smooth muscle relaxant activity was measured by inhibition of calcium contractures of potassium-depolarised isolated guinea-pig ileum.[16] Calcium (2.5 mM) induced a sustained contraction of guinea-pig ileum which was inhibited by 48.1 ± 2.3% by nifedipine (1×10^{-8} M). In contrast, **6** and **7** (1×10^{-5} M) inhibited contractures by 18.9 ± 2.16% and 36.37 ± 5.7%, respectively (Table 1). The difference between the two diastereoisomers was not statistically different ($P > 0.05$). It is unlikely that such a degree of smooth muscle relaxation would be clinically useful.

Following our original observation that similar compounds inhibited calcium contractions of smooth muscle, probably by inhibition of calcium handling in excitation–contraction coupling in smooth muscle cells[6] we had extended our studies to encompass a potential role in excitation–secretion coupling in secretory cells,



Scheme 1. Synthetic route to compounds **6** and **7**.

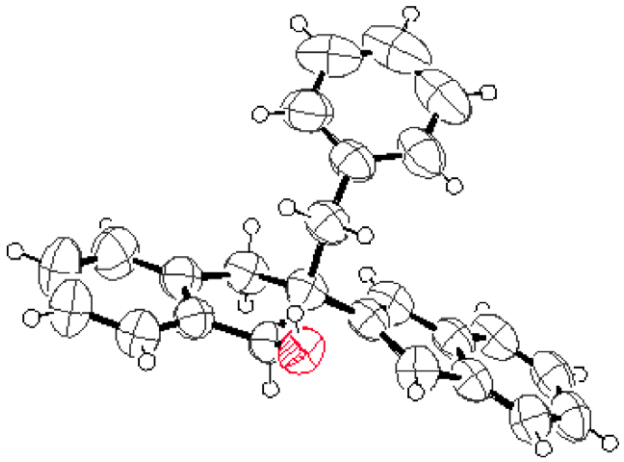


Figure 1. X-ray crystal structure and relative stereochemistry of **6**.

Table 1
Effect of nifedipine (1×10^{-8} M), and compounds **6** and **7** (1×10^{-5} M) on calcium-induced (2.5 mM) contractions of potassium-depolarised guinea-pig ileum

Treatment	Mean	SEM	*n*
Nifedipine	48.1	2.3	6
6	18.88	2.16	6
7	36.37	5.667	12

as histamine release inhibitors in rat peritoneal mast cells.[10] In addition, we have used the histamine-releasing rat cell line RBL-2H3 as a convenient alternative to rat peritoneal mast cells harvested by peritoneal lavage.

Histamine release[17] from rat peritoneal mast cells stimulated by compound 48/80 was inhibited by disodium cromoglycate by only approximately 11% (Table 2). However, **6** and **7** both inhibited histamine release in a much more potent manner, by 84% and 93% from 60.2 ± 5.0% for 48/80-stimulated release to 9.3 ± 1.3% and 4.1 ± 0.4%, respectively, the difference between the two not being statistically different ($P > 0.05$). Similarly, both **6** and **7** (10 μM) inhibited calcium ionophore A23187-stimulated β-hexosaminidase release from RBL-2H3 cells,[17–19] by approximately 45% and 40%, from a solvent (DMSO) control value of 35.2 ± 4.8% to 19.2 ± 3.2% and 21.0 ± 2.8%, respectively (Fig. 2). In comparison, quercetin (10 μM) inhibited enzyme release by approximately 70% to 11.0 ± 1.0% of the total as shown in Figure 3. Furthermore, such inhibition was dose-dependant; concentrations of both **6** (3–50 μM) and **7** (3–50 μM) gave a highly significant ($P < 0.0001$) linear trend, with maximum inhibition at 50 μM being 96% and 75%, from a solvent (DMSO) control value of 80.5 ± 9.4% to 20.1 ± 0.2% and 3.2 ± 0.4% for **6** and **7**, respectively, though there was no significant ($P > 0.05$) difference between **6** and **7** at this concentration.

These results suggest that both **6** and **7** are inhibitors of mediator release from RPMCs and RBL-2H3 cells. Furthermore, in the case of the latter, mediator release is dose-dependant. However,

Table 2
Histamine release from rat peritoneal mast cells stimulated by compound 48/80 (2 μg mL^{-1}) and the effect of DSCG (2×10^{-5} M) and compounds **6** and **7** (2×10^{-5} M)

Treatment	Mean	SEM	*n*
48/80 alone	60.23	4.984	15
DSCG	53.62	4.009	15
6	9.339	0.4251	5
7	4.138	1.316	5

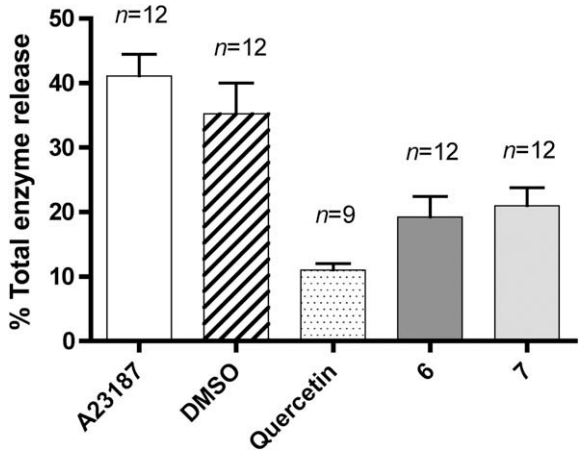


Figure 2. Effect on β-hexosaminidase release from RBL-2H3 cells stimulated by calcium ionophore A23187 (5 μg/mL) by quercitin (10 μM), compounds **6** and **7** (10 μM). The value obtained on incubation with the solvent control, DMSO (0.5%) is shown for comparison. Values are expressed as a mean ± SEM, *n* as indicated.

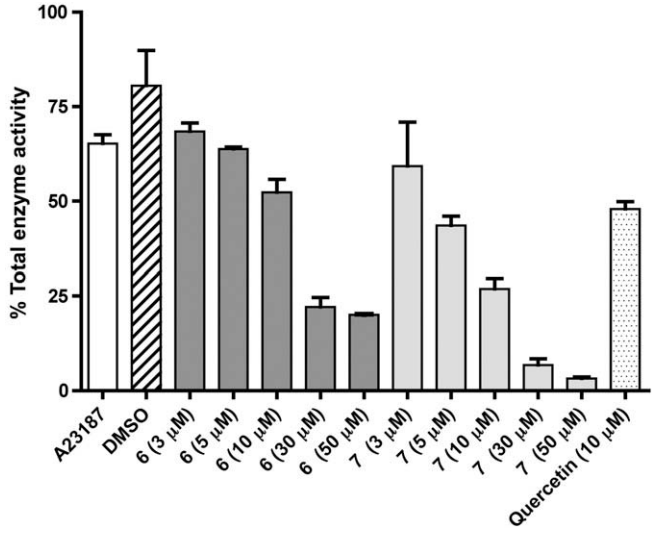


Figure 3. The concentration effect relationship of compounds **6** and **7** (3–50 μM) on β-hexosaminidase release from RBL-2H3 cells stimulated by calcium ionophore A23187 (5 μg/mL). The value obtained on incubation with the solvent control, DMSO (0.5%) and by quercetin (10 μM) is shown for comparison. Values are expressed as a mean ± SEM, $n = 3$.

we have previously shown in preliminary studies that both **6** and **7** were largely ineffective against IgE-mediated release of histamine and β-hexosaminidase from RBL-2H3 cells.[20] While the fact that the compounds seem only to be effective against non-immunogenic stimuli may mitigate against an anti-allergic application of these compounds, their mediator releasing inhibitory effects may be applicable to inflammation in general.

The arachidonic acid mouse ear oedema model is useful to screen potential anti-inflammatory compounds with an uncertain mode of action, since it encompasses a wide range of potential targets.[21] Application of arachidonic acid (4 mg) to a mouse ear gives rise after 1 h to an increase in ear thickness of 78.8 ± 14.2%. Prior application (1 h) of indomethacin (300 μg) reduced ear swelling by 50%, to 39.6 ± 5.0%. In comparison, **6** and **7** (300 μg) reduced ear swelling by approximately 45% and 59% to 43.0 ± 7.5% and 32.4 ± 6.8%, respectively (Table 3), the difference between the two compounds, **6** and **7** not being statistically different ($P > 0.05$). The efficacy of both **6** and **7** was comparable to that of

Table 3
Effect of vehicle only, indomethacin (300 μg), and **6** and **7** (300 μg) on arachidonic acid-induced (4 mg) mouse ear swelling

Treatment	Mean	SEM	*n*
Vehicle	178.8	14.21	8
Indomethacin	139.7	5.035	4
6	143.1	6.797	4
7	132.4	7.493	4

All treatments were administered topically in 100 μL acetone.

the positive control, indomethacin, suggesting that the compounds may have anti-inflammatory activity in vivo, with possible clinical potential. Further experiments should follow systemic administration of **6** and **7**, to exclude the possibility of topical interaction between the compounds and arachidonic acid.

Conclusion. In conclusion, we report the synthesis and activity of a pair of diastereomeric indane dimers that demonstrate inhibition of histamine release from mast cells and demonstrate dose dependent inhibition of β-hexosaminidase release from RBL-2H3 cells. In addition these compounds demonstrate anti-inflammatory activity in vivo in the arachidonic acid mouse ear model. In a preliminary study,[22] while we showed that IgE-stimulated histamine release from RBL-2H3 cells was largely unaffected by the compounds, we speculated that greater biological activity might reside in diastereoisomer **7**, on the basis that the marginal effect did attain statistical significance. However, we have been unable to confirm any such difference in the activity of the two diastereoisomers in this study; both **6** and **7** were equipotent in all systems studied. Furthermore, we have shown significant anti-inflammatory activity in vivo and these molecules are currently under investigation in other inflammatory disease models as potential novel therapeutic agents.

References and notes

1. Metz, M.; Grimbaldeston, M. A.; Nakae, S.; Piliponsky, A. M.; Tsai, M.; Galli, S. J. *Immunol. Rev.* **2007**, *217*, 304.
2. Puxeddu, I.; Piliponsky, A. M.; Bachelet, I.; Levi-Schaffer, F. *Int. J. Biochem. Cell. Biol.* **2003**, *35*, 1601.
3. Krishnaswamy, G.; Ajitawi, O.; Chi, D. S. *Methods. Mol. Biol.* **2006**, *315*, 13.
4. De Clercq, E. *Int. J. Antimicrob. Agents* **2009**, *33*, 307.
5. Abrams, W.; Irvin, J.; Tobert, J.; Ferguson, R.; Vlasses, P. *Cardiovasc. Drug Rev.* **2007**, *3*, 1.
6. Leoni, L.; Hamel, E.; Genini, D.; Shih, H.; Carrera, C. J.; Cottam, H. B.; Carson, D. A. *J. Natl. Cancer Inst.* **2000**, *92*, 217.
7. Sheridan, H.; Lemon, S.; Frankish, N.; McCardle, P.; Higgins, T.; James, J.; Bhandari, P. *Eur. J. Med. Chem.* **1990**, *25*, 603.
8. Farrell, R.; Kelleher, F.; Sheridan, H. *J. Nat. Prod.* **1996**, *59*, 446.
9. Sheridan, H.; Frankish, N.; Farrell, R. *Eur. J. Med. Chem.* **1999**, *34*, 953.
10. Sheridan, H.; Frankish, N.; Farrell, R. *Planta Med.* **1999**, *65*, 271.
11. Frankish, N.; Farrell, R.; Sheridan, H. *J. Pharm. Pharmacol.* **2004**, *56*, 1423.
12. Sheridan, H.; Walsh, J. J; Jordan, M.; Cogan, C.; Frankish, N. *Eur. J. Med Chem.*, submitted for publication.
13. *Compound* ***8***: Triethylamine (12.7 mL, 90.8 mmol) followed by trimethylsilyltrifluoromethane sulfonate (TMS triflate) (15.0 mL, 83.3 mL), was added slowly dropwise at 0 °C, to a stirred solution of 1-indanone (10.00 g, 75.7 mmol) in CH_2Cl_2 (150 mL) at 0 °C. After the addition, the solution was allowed to warm to rt and stirred for 20 min before being cooled to 0 °C and quenched by the addition of 5% aq. $NaHCO_3$ (150 mL). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL), the combined organic extracts were washed with water and brine, dried over $MgSO_4$ and evaporated. The crude product was filtered through a short silica pad and washed through with 100:0.5 hexane/ethyl acetate. The solvent was again removed in vacuo with a bath temperature of not more than 40 °C, to give 15.25 g (98%) of the product as a pale yellow oil. ^1H NMR analysis was not possible due to decomposition of the product over time in the NMR solvent. ν_{max} (neat): 3072, 3075, 2954, 2895, 1607, 1578, 1350, 1255 (Si–Me_3), 1178, 1125 (Si–O), 1078, 884 (Si–O), 849 (Si–Me_3), 755 (C–H_{Ar}). *Compound* ***9***: Trimethyl orthoformate (20.7 mL, 189.2 mmol) and catalytic *p*-toluenesulfonic acid (0.15 g, 1 mol %) were added to a solution of 2-indanone (10.42 g, 78.8 mmol) in methanol (125 mL). The solution was allowed to stir at room temperature for 2 h and then quenched by the addition of solid $NaHCO_3$ (0.5 equiv). The methanol was evaporated in vacuo and the crude material partitioned between water and ether (ca. 100 mL each). The layers were separated and the aqueous layer extracted with ether (2 × 100 mL). The

combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (98:2 hexane/ethyl acetate) δ_H (300 MHz, $CDCl_3$): 3.14 (4H, s, 2 × CH_2), 3.29 (6H, s, 2 × CH_3), 7.12–7.19 (4H, m, ArH). *Compound* **11**: Benzyl bromide (1.9 mL, 16 mmol) was added to a stirred solution of **10** (1.13 g, 4 mmol) in ether (34 mL) and *tert*-butanol (6 mL). Potassium *tert*-butoxide (0.45 g, 4 mmol) in *tert*-butanol (20 mL) was added drop wise and reaction was stirred for 3 h. The reaction was quenched by the addition of satd NH_4Cl and the layers separated. The reaction was worked up and the crude product was purified by flash chromatography (9:2; hexane/ethyl acetate). δ_H (300 MHz, $CDCl_3$): 3.37 (2H, dd, *J* 13.8 Hz, CH_2), [AB system], 3.38–3.61 (4H, m, 2 × CH_2), 6.70 (1*H*, s, CH═C), 7.09–7.34 (10H, br m, ArH), 7.36–7.39 (1*H*, m, ArH), 7.50–7.55 (1*H*, m, ArH), 7.91 (1*H*, d, *J* 7.7 Hz, ArH). *Compounds* ***6*** *and* **7**: Lithium tri-*tert*-butoxyaluminohydride (11.57 g, 45 mmol) was added in one portion to a stirred solution of the **11** (4.64 g, 14 mmol) in THF (115 mL). The solution was stirred at room temperature and monitored by TLC (9:1 hexane/ethyl acetate). After work up the organic extract was evaporated to yield a pale yellow oil (85%) containing of **6** and **7** (1:1). The diastereoisomers were separated by flash chromatography using a *n*-hexane: ethyl acetate gradient. *Compound* ***6***: δ_H (300 MHz, $CDCl_3$) 2.51 (1*H*, d, *J* 5.3 Hz, OH), 2.94 (2H, dd, *J* 13.4 Hz, CH_2) [AB system], 2.98 (2H, dd, *J* 15.6 Hz, CH_2) [AB system], 3.46 (2H, dd, *J* 22.5 Hz, CH_2) [AB system], 5.17 (1*H*, d, *J* 5.1 Hz, C*H*OH), 6.44 (1*H*, s, C*H*═C), 6.82–6.85 (2H, m, ArH), 7.09–7.14 (4H, m, ArH), 7.22–7.26 (5H, m, ArH), 7.36–7.39 (2H, m, ArH). *Compound* **7**: δ_H (300 MHz, $CDCl_3$):3.00 (2H, dd, *J* 13.8 Hz, CH_2) [AB, centered at 3.00], 3.08 (2H, dd, *J* 15.8 Hz, CH_2) [AB system], 3.41 (2H, dd, *J* 22.9 Hz, CH_2) [AB system], 5.02 (1*H*, m, C*H*OH), 6.64 (1*H*, s, C*H*═C), 6.91–6.94 (2H, m, ArH), 7.10–7.31 (9H, br m, ArH), 7.35–7.40 (2H, m, ArH).

14. Sheridan, H.; Walsh, J. J.; McCabe, T.; Passante, E.; Cogan, C.; Jordan, M.; Frankish, N., *X-ray crystal data deposit*. CCDC deposition number: 733630, 2009.
15. Passante, E.; Carsten, E.; Sheridan, H.; Frankish, N. *Inflamm. Res.* **2009**, *58*, 611.
16. *Smooth muscle relaxant activity:* Measurement of smooth muscle relaxant activity was performed as previously described.[6]
17. *Histamine release:* Measurement of inhibition of inhibition of histamine release from rat peritoneal mast cells was carried out as decribed previously.[9]
18. RBL-2H3 Cell culture; RBL-2H3 cells were purchased from LCG Promochem (Teddington, UK) and were cultured in α-MEM (GIBCO, Paisley, UK) supplemented with 10% foetal bovine serum (FBS), (Biosera, Ringmer, UK) and 100 U/mL penicillin–streptomycin (GIBCO) at 5% CO_2 and 37 °C. Cells were subcultured using either trypsin–EDTA (GIBCO) or Accutase® (Bioquote, York, UK) when reaching 80% confluency and were plated at 1 × 10^5 cells/cm^2 in 24-well plates for experiments.
19. *β-Hexosaminidase assay:* RBL-2H3 cells (1 × 10^5 cells/cm^2) were seeded and they were let to adhere for at least 3 h. Then the supernatant was removed and replaced with fresh medium containing calcium ionophore at 5 μg/mL and test compounds at the desired concentration (0.3–50 μM). Plates were incubated for 30 min at 5% CO_2 and 37 °C. The β-hexosaminidase activity was assayed according to a previously published method, [2]. Briefly, 30 μL of supernatant were transferred into a 96-well plate. Fifty microliters of substrate solution were added (1.3 mg/mL of *p*-nitrophenyl *N*-acetyl-D-glucosaminide (Sigma) in citrate buffer, pH 4.5) and the plate was incubated for 1 h at 37 °C. The reaction was stopped by adding 80 μL of NaOH (0.5 M) to each well and the formed *p*-nitrophenolate was measured spectrophotometrically in a 96-well plate reader (FLUOstar OPTIMA, BMG Labtech, Aylesbury, UK) at a wavelength of 405 nm. The absorption was converted into the percentage of total cellular β-hexosaminidase activity by comparison with the absorption produced by a Triton X-100 (Sigma) lysate of the same cells according to the following equation:% *total enzyme activity* = [(*secreted* − *spontaneous*)/*total content*] * 100. Incubations were performed in triplicate. The percentage of the vehicle was always maintained at 0.5% of the volume of the supernatant.
20. Passante, E.; Carsten, E.; Sheridan, H.; Frankish, N. *FASEB J.* **2007**, *21*, A442.
21. *Mouse ear oedema:* The mouse ear oedema model was performed using Balb/C mice (25–35 g), of either sex. The left ear was treated by the topical application (10 μL) of solvent (acetone 100%). To the right ear was applied 10 μL test compound (300 μg/ear in acetone), indomethacin (300 μg/ear in acetone) or dexamethasone (100 μg/ear in acetone). After 1 h, oedema was induced by the topical application of arachidonic acid (10 μL of 400 mg/mL in acetone). The width of each ear was measured, both before and 60 min after the induction of oedema, using a micrometer screw gauge. Ear oedema was calculated by comparing the left and right ear width after induction of oedema and expressed as percentage normal. Values are expressed as a mean ± SEM and statistical comparisons between groups was performed by one way Anova, followed by Dunnet's 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.
22. Passante, E.; Ehrhardt, C.; Sheridan, H.; Frankish, N. *Inflamm. Res.* **2008**, *57*, 15.