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Title

A one-pot and eco-friendly synthesis of novel β-substituted-α-halomethyl acrylates and bioactivity of these compounds in an *in vitro* model of mast cell degranulation induced by pro-inflammatory stimuli

Authorship

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Abstract

The main goal of the present work was to develop new compounds derived from β-substituted-α-halomethyl acrylates from a methodology in an aqueous phase and to evaluate their bioactivity as potential inhibitors of mast cell activation. Adult male Wistar rats (n=205) were used. Mast cells were obtained by peritoneal lavage and purified in a discontinuous gradient of Percoll. Compound 48/80 (10 µg/ml) and the calcium ionophore A23187 (0.12 µg/ml) were used as mast cell secretagogues. Different concentrations of the 11 synthetic drugs (10 to 320 µM) were used. The release of β-hexosaminidase was quantified as a marker of mast cell activation. Mast cell morphology was evaluated by light and electron microscopy. Comparison studies with a reference compound, sodium chromoglycate, were also made. EC50 was calculated. Statistical analysis: ANOVA-1/Tukey-Kramer. Enzymatic release under basal conditions showed values below 10 %. Compounds 48/80 and A23187 significantly stimulated the release of β-hexosaminidase from mast cells. The effect induced by compound 48/80 was inhibited by compound **5** (320 µM) and compound **9** (160 and 320 µM) without causing cytotoxic effects. The effect induced by A23187 was inhibited by compound **5** (40, 80, 160, and 320 µM) without affecting cell viability. The inhibitory effects exhibited by compounds **5** and **9** were more potent than that of the reference compound. The biochemical results were consistent with the morphological findings. This study reports, for the first time, that the new synthetic compounds methyl (*Z*)-2-bromo-3-(furan-3-yl)acrylate (compound **5**) and methyl (*E*)-2-bromo-3-(3-bromophenyl)acrylate (compound **9**) strongly inhibit mast cell degranulation, without affecting cell viability. The implications of these results are relevant as a basis for developing new anti-inflammatory and mast cell stabilizing drugs.

Keywords

Horner-Wadsworth-Emmons reaction; mast cells; compound 48/80; calcium ionophore A23187; β-hexosaminidase; degranulation

1. Introduction

There is a growing interest, in the field of medicinal chemistry and experimental pharmacology, in the development of molecular structures capable of regulating cellular activity, such as anti-proliferative (Romagnoli et al., 2017; Song et al., 2017), cytoprotective (Gu et al., 2019), antiparasitic (Ettari et al., 2016), antitumor (Magalhaes et al., 2018), and anti-inflammatory agents (Lukan, 2020; Yeung et al., 2018). For this reason, efforts are being made to design compounds containing substructures with α,β-unsaturated carbonyl groups, capable of acting as Michael acceptors against nucleophilic cellular substructures (Romagnoli et al., 2010). Proteins and nucleic acids possess hydroxyl, amino, and thiol groups capable of acting as nucleophiles. So, cell physiology could be regulated due to this molecular interaction between nucleophiles and α,β-unsaturated carbonyls (Gehringer and Laufer, 2019; Jackson et al., 2017).

Previously, our research group reported the synthesis of cyclic α,β-unsaturated esters derived from furan, called butenolides (Ceñal et al., 2005). Together with others purified from plants, these synthetic structures were evaluated biologically in an *in vitro* model of rat peritoneal mast cell activation and in an *in vivo* model of gastric ulcer induced by mast cell activation. We have shown that these substances act as pharmacological regulators of mast cell activation and, more specifically, as mast cell stabilizers (Coll et al., 2020; Penissi et al., 2009; Vera et al., 2021, 2012).

Mast cells are a significant component of innate and adaptive immunity (Arthur and Bradding, 2016). These connective tissue cells are critical players of the inflammatory response and have been involved in a large number of processes, either protective (tissue homeostasis and wound healing) or damaging (chronic inflammation, gastric ulcer, cancer, autoimmune diseases) to the organism (Bulfone-Paus et al., 2017; Falduto et al., 2021; Frossi et al., 2018; Giannetti et al., 2021; Monticelli and Leoni, 2017; Varricchi et

al., 2019). Mast cells are increasingly attractive targets for natural and synthetic products due to the increasingly important role of these cells in both physiological and pathological conditions (Zhang et al., 2016).

A comparative study of the mast cell stabilizing activity of our natural and synthetic compounds showed that the pharmacological potency of the butenolides was lower than that of the other molecules tested, including pharmacological reference compounds (Coll et al., 2020; Penissi et al., 2009; Vera et al., 2021, 2012). Furthermore, structural analysis of the butenolides shows that these molecules have an α,β-unsaturated endocyclic bond instead of the α,β-unsaturated exocyclic double bond of other compounds with greater pharmacological potency, such as dehydroleucodine and xanthatin (Coll et al., 2020; Penissi et al., 2009; Vera et al., 2021, 2012). This functional and conformational arrangement suggests a possible molecular steric hindrance in the butenolides, thereby conditioning their interaction at the cellular level and reducing their biological potency. So, based on the described background, we proposed to design new synthesis compounds through an environmentally friendly methodology oriented to synthesizing structures with double bonds conjugated to a carbonyl group in alicyclic molecular designs.

The main goal of the present work was to develop new compounds derived from β-substituted-α-halomethyl acrylates from a methodology in an aqueous phase and to evaluate their bioactivity as potential inhibitors of mast cell activation.

2. Materials and Methods

2.1. Chemicals and reagents

Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates (Kiesel gel 60F254 0.25 mm). Column chromatography was

performed with Silica Gel 60 (0.04-0.063 mm/230-400 mesh) ASTM produced by Macherey-Nagel GmbH and Co. The organic solvent was dried using a vacuum rotary evaporator (BUCHI Water Bath B-480). Analysis with GC-MS was performed with a Thermo ITQ-900 that included AutoSampler AS-1310, capillary column OV-5MS, 30 m, 0.25 mm of internal diameter, 0.25 µm of film thickness, chemically bound and cross-linked. ^{1}H and ^{13}C-RMN analysis were performed with a Bruker Avance 200 MHz spectrometer equipped with a BBI 200 MHz S1 5 mm reverse detection probe with a magnetic field gradient on the z-axis. The spectra were processed and analyzed with the TopSpin 2.0 program. Chemical shifts (δ) were reported in parts per million (ppm) downfield from tetramethylsilane (δ 0.00) or $CHCl_3$ (δ 7.26) for ^{1}H-NMR and δ 77.0 for ^{13}C-NMR as internal standard and coupling constants were reported in Hertz. The IR spectra were obtained using the potassium bromide tablet technique with Fourier Transform Infrared Spectrometer (FTIR), Nicolet Protege 460: measuring range: 4000 a 250 cm^{-1}, sweep number: 64, resolution: 4 cm^{-1}. The melting point was obtained with a hot plate melting point microscope Leitz Wetzlar Germany 553174.

Compound 48/80 and calcium ionophore A23187 were dissolved in dimethyl sulfoxide (DMSO) and stored at –80ºC until required. Then, the stock solutions were diluted with phosphate-buffered saline solution (PBS) to the final concentration (10 µg/ml compound 48/80 and 0.12 µg/ml for A23187). β-substituted-α-halomethyl acrylates were dissolved in DMSO (stock solutions: 100 mg/ml) and stored at –80ºC until required. Next, the stock solutions were diluted to the desired final concentration with PBS. The final concentration of DMSO in reagent media was less than or equal to 1%. Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

2.2. Synthesis of 3-substituted-2-bromo-methyl acrylates and 3-substituted-2-iode-methyl acrylates

3-substituted-2-bromo-methyl acrylates and 3-substituted-2-iode- methyl acrylates were synthesized through a modified Horner-Wadsworth-Emmons (HWE) reaction to obtain the trisubstituted alkenes (Roman et al., 2021). Fig. 1 shows a scheme of this reaction. Briefly, trimethyl phosphonoacetate (0.5 ml, 3 mM) (Figure 1, part 1) and Na_2CO_3 (318 mg, 3 mM) were dispersed in water (30 ml) with magnetic agitation at 0°C. Immediately, 1.5 mM NBS or NIS (depending on the reaction) was added slowly, in small portions, for 30 min. Substituted aromatic aldehydes were added (1.5 mM) (Figure 1, part 2). THF (tetrahydrofuran) (1.0 ml) was a good cosolvent for this procedure. TLC monitored the progress of the reaction. After 24 or 48 h at room temperature, the reactions were quenched by adding 0.1 N HCl, pH = 7, and extracted with ethyl ether (3 x 10 ml) three times. The organic layer was washed with water (2 x 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated under a vacuum to remove the ethyl ether. The residue was purified by flash chromatography on silica gel using ethyl acetate and hexane (10:90) as eluent solvent. All products (Figure 1, part 3) were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and GC-MS.

2.3. Animals

Wistar adult rats (n = 226) weighing approximately 300 to 500 g, infection-free, and maintained under a 12-hour dark/light cycle in a temperature-controlled room (24-25ºC) with free access to drinking water and laboratory food were used.

2.4. Ethics statement

All animal experiments were evaluated and approved by the Institutional Committee for Care and Use of Laboratory Animals (IACUC), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo (Protocol No. 182/2020). Regulations of this Committee are in strict accordance with the recommendations in the Guide for the Care and Use of

Laboratory Animals of the National Institute of Health (NIH, USA) to comply with established international regulations and guidelines.

2.5. Isolation and purification of mast cells

Mast cells were isolated by peritoneal lavage as previously described (Berdún et al., 2015) with some modifications (Coll et al., 2020). Peritoneal mast cells were purified by centrifugation through a discontinuous gradient of Percoll (Inagaki et al., 2001). Cells were stained with toluidine blue (0.1% w/v, pH 1.0) and quantified using a Neubauer hemocytometer under a Nikon microscope (magnification 400X). Peritoneal cell suspensions contained 3% mast cells, and the purity of the mast cells after gradient centrifugation was more than 90%. Purified mast cells were washed, resuspended in a balanced salt solution (cell density of 2 x 10^6/ml), and maintained for 30 min at 4ºC. Mast cells were considered viable upon their ability to exclude trypan blue and the levels of β-hexosaminidase released to the supernatants. The trypan blue-exclusion test indicated viability of over 95%. In all experiments, the nonspecific, spontaneous β-hexosaminidase release was less than 10%.

2.6. Bioactivity of β-substituted-α-halomethyl acrylates in a mast cell degranulation assay

Purified peritoneal mast cells (100 µl; cell density 1 x 10^6/ml) were preincubated in polypropylene tubes for 10 min at 37ºC in the presence of the test compounds (each of the 11 β-substituted α-halo-β-substituted methyl acrylates obtained), before incubation with balanced salt solution or compound 48/80 (final concentration 10 µg/ml) or calcium ionophore A23187 (final concentration 0.12 µg/ml) or for an additional 10 min at 37ºC. Dose-response dependence studies were conducted using concentrations of β-substituted α-halo-β-substituted methyl acrylates of 10, 20, 40, 80, 160, and 320 µM. Negative (no stimulation with compound 48/80 or calcium ionophore A23187) and positive

(stimulation with compound 48/80 or calcium ionophore A23187) controls were included in all the experiments. Comparative studies with sodium cromoglycate, a classical mast cell stabilizer, were also made within the same concentration and time ranges. The mean total number of mast cells during incubations was 4 x 10^4/ml *per* tube. The secretion was stopped by cooling the tubes in an ice-cold water bath. Cells and supernatants were separated by centrifugation (180 g, 5 min, 4ºC). The supernatants were used to determine the β-hexosaminidase content by colorimetric reaction, taken as a measure of β-hexosaminidase release. The cell pellets were lysed with 1% Triton X-100 to liberate the residual β-hexosaminidase, quantified by colorimetric reaction, and taken as a measure of the remaining β-hexosaminidase. Other samples of cell pellets were used for cell viability studies by the trypan blue dye exclusion test, fixed and stained with toluidine blue for light microscopy and morphometry studies, or fixed and processed for transmission electron microscopy. Cell viability studies were carried out to ensure that changes in the β-hexosaminidase release were not due to cell death. The percentage of β-hexosaminidase release in each tube was calculated. All the experiments were repeated at least five times.

2.7. Quantification of β-hexosaminidase levels

β-hexosaminidase release, an index of mast cell degranulation, was determined using a colorimetric assay as previously reported (Puri and Roche, 2008) with some modifications. Briefly, an aliquot of the supernatant was mixed with an equal volume of 2 mM substrate solution (p-nitrophenyl-N-acetyl-β-D-glucosaminide in 0.2 M citrate, pH 4.5) and then incubated for 3 h at 37 °C. The reaction was stopped by adding 0.4 M glycine in NaOH, pH 9. Absorbance was measured with a microplate reader at 405 nm (Thermo Scientific Multiskan FC, Helsinki, Finland). Results were expressed as the

percentage of β-hexosaminidase activity released over the total (enzyme released plus intracellular enzyme).

2.8. Light microscopy and morphometry

Mast cells were fixed in 3% glutaraldehyde. After 2 h in the fixative, cell suspensions (2 x 10^5/ml) were stained with toluidine blue (0.1% w/v, pH 3.0), placed between slides and cover slides, and examined under a Nikon 80i microscope. The percentage of mast cells exhibiting degranulation was quantitated at a magnification of 400X. Mast cell degranulation was defined as extruded granules close to the cell's surface, scalloped edges, or staining of about half or less of the cell with toluidine blue.

2.9. Transmission electron microscopy

The transmission electron microscopy procedure was performed as previously described (Persia et al., 2014).

2.10. Statistical analysis

Results from biochemical and morphometric studies are presented as means ±SEM. Differences between groups were determined using analysis of variance followed by the Tukey-Kramer multiple comparisons test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Synthesis of 3-substituted-2-bromo-methyl acrylates and 3-substituted-2-iode-methyl acrylates

A series of 3-substituted-2-bromo-methyl acrylates and 3-substituted-2-iode- methyl acrylates were prepared according to the synthetic route shown in Figure 1. The chemical name, structure, molecular weight, state of aggregation, melting point, and reaction time of each molecule are shown in Table 1.

These compounds were characterized by proton nuclear magnetic resonance (^{1}H-NMR), carbon nuclear magnetic resonance (^{13}C-NMR), mass spectrometry, and infrared analysis. Spectral data are detailed below.

- *Compound 1:* IR: υ= 3014, 2921, 1720, 1590, 1430, 1520, 1350 cm-1; 1H-RMN (200 MHz, CDCl3): δ = 8.21 (d, J= 8.6 Hz, 2H), 7.44 (d, J= 8.4 Hz, 2H), 7.44 (s, 1H), 3.77 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ = 163.83, 147.59, 141.03, 138.13, 128.91, 128.91, 123.68, 123.68, 115.11, 53.31; GC-MS: M+ = 286, m/z 207, 206, 174, 75.
- *Compound 2:* IR: υ = 3020, 2960, 1720, 1590, 1520, 1350, 1270 cm-1; 1H-RMN (200 MHz, CDCl3): δ= 8.28 (d, J = 8.8 Hz, 2H), 8.26 (s, 1H), 7.96 (d, J = 8,6 Hz, 2H), 3.94 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ = 163.09, 148.05, 139.96, 138.57, 130.72, 130.72, 123.58, 123.58, 116.76, 53.89; GC-MS: M+ =286, m/z 207, 206, 174, 75.
- *Compound 3:* IR: υ = 2950, 1730, 1600, 1510, 1220 cm-1; 1H-RMN (200 MHz, CDCl3): δ = 7.33 (d, J = 9.0 Hz, 1H), 7.30 (d, J = 2.6 Hz, 1H), 7.30 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.78 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ= 165.32, 164.49, 160.35, 139.30, 130.27, 130.10, 115.71, 115.28, 110.95, 52.98; GS-MS: M+ = 259, m/z 179, 147, 120.
- *Compound 4:* IR: υ= 3010, 2950, 1720, 1600, 1510, 1230 (R-F); 1H-RMN (200 MHz, CDCl3): δ= 8.18 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.09 (d, J = 8.6 Hz, 1H), 3.90 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ= 166.00, 163.73, 160.99, 139.80, 132.55, 132.38, 115.81, 115.37, 112.19, 53.56; GC-MS: M+ = 259, m/z 179, 147,120.
- *Compound 5:* IR: υ= 3140, 3030, 1745, 1630, 1430; 1H-RMN (200 MHz, CDCl3): 8.13 (bs, 1H), 8.10 (bs, 1H), 7.48 (s, 1H), 7.01 (s, 1H), 3.88 (s, 3H); 13C-

RMN (50 MHz, CDCl3): δ= 163.67, 151.10, 146.36, 143.36, 132.56, 121.03, 110.63, 53.41; GS-MS: M+ = 231, m/z 151, 119, 92, 63.

- *Compound 6:* IR: υ= 3010, 2950, 1710, 1610, 1490, 517; 1H-RMN (200 MHz, CDCl3): δ= 7.31 (d, J = 8.2 Hz, 2H), 7.29 (s, 1H), 7.21 (d, J = 8.4 Hz, 2H), 3.76 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ= 164.40, 139.10, 134.94, 133.10, 129.46, 129.46, 128.65, 128.65, 111.75; 53.05; GS-MS: M+ = 275, m/z 195, 163, 136.
- *Compound 7:* IR: υ= 3040, 2950, 1720, 1610, 1490, 517; 1H-RMN (200 MHz, CDCl3): δ= 8.17 (s, 1H), 7.79 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 3.90 (s, 3H); 13C-RMN (50MHz, CDCl3): δ= 163.61, 139.72, 136.17, 132.02, 131.50, 131.50, 129.13, 128.71, 112.5, 53.64; GS-MS: M+ = 275, m/z 195, 163, 136.
- *Compound 8:* IR: υ= 2990, 2950, 1700, 1590, 1520, 1430-1340, 719; 1H-RMN (200 MHz, CDCl3) δ= 8.20 (d, J = 8.6 Hz, 2H), 7.59 (s, 1H), 7.41 (d, J = 8.4 Hz, 2H), 3.76 (s, 3H); 13C-RMN (50 MHz, CDCl3) δ= 165.74, 147.51, 144.61, 142.36, 128.54, 128.54, 123.74, 123.74, 88.27, 53.37; GS-MS: M+ = 333, m/z 206, 175, 101, 89, 75.
- *Compound 9:* IR: υ= 3050, 3010, 1710, 1610, 1560, 681; 1H-RMN (200 MHz, CDCl3) δ= 7.44 (d, J =11.6 Hz, 2H), 7.3 (s, 1H), 7.20 (d, J = 5 Hz, 2H), 3.77 (s, 3H); 13C-RMN (50MHz, CDCl3) δ= 164.29, 138.47, 136.57, 131.82, 130.93, 129.88, 126.59, 122.36, 112.68, 53.09; GS-MS: M+= 320, m/z 240, 180, 101, 75.
- *Compound 10:* IR: υ= 3000, 2950, 1720, 1610, 1480, 501; 1H-RMN (200 MHz, CDCl3): δ= 7.46 (d, J = 8.4 Hz, 2H), 7.30 (s, 1H), 7.15 (d, J = 8.4 Hz, 2H), 3.76 (s, 3H); 13C-RMN (50 MHz, CDCl3): δ= 164.36, 139.10, 133.48, 131.50, 131.50, 129.64, 129.64, 123.19, 111.79, 53.05; GS-MS: M+= 320, m/z 240, 180, 101, 75.
- *Compound 11:* IR: υ= 3000, 2950, 1720, 1600, 1510, 1220; 1H-RMN (200 MHz, CDCl3): δ= 7.48 (s, 1H), 7.25 (d, J= 8.8 Hz, 2H), 7.02 (d, J= 8.8 Hz, 2H), 3.75 (s,

3H); 13C-RMN (50 MHz, CDCl3): δ= 165.33, 160.36, 145.78, 132.48, 129.92, 129.75, 115.79, 115.36, 83.33, 53.12; GS-MS: M+= 306, m/z 179, 147, 120, 74.

The highest yields were observed for methyl (*E*)-2-bromo-3-(4-nitrophenyl)acrylate, methyl (*Z*)-2-bromo-3-(4-nitrophenyl)acrylate, methyl (*E*)-2-bromo-3-(4-chlorophenyl)acrylate, and methyl (*Z*)-2-bromo-3-(4-chlorophenyl)acrylate (compounds 1, 2, 6 and 7, respectively). 96 % of the total product in each reaction was observed. For compounds 3 and 4, the total yield was 86%, obtaining almost the same proportion for each isomer. In every case, the *E* isomer was the first to leave the chromatographic column and, therefore, the least polar isomer. For the rest of the compounds (5, 8, 9, 10, and 11), obtaining both pure stereoisomers was not possible.

3.2. Bioactivity of β-substituted-α-halomethyl acrylates in a mast cell degranulation assay

3.2.1. Effect of β-substituted-α-halomethyl acrylates on mast cell β-hexosaminidase release induced by compound 48/80

The effect of each of the 11 new synthetic compounds on mast cell β-hexosaminidase release induced by compound 48/80 was analyzed (Fig. 2). In addition, the action of each of these treatments on cell viability was evaluated (Fig. 3). In all cases compound 48/80 stimulated the release of β-hexosaminidase from mast cells. This effect was inhibited by compound **5** at the concentration of 320 µM and by compound **9** at the concentrations of 160 and 320 µM. These effects were not due to cytotoxic effect of the tested compounds. None of the 11 new synthetic compounds *per se* modified the basal release of β-hexosaminidase.

To analyze the effect of compound **5** and compound **9** on mast cell activation induced by a secretagogue acting by mechanisms other than compound 48/80, the action of these new molecules on mast cell degranulation induced by the calcium ionophore A23187 was

studied (Fig. 4). In both cases the ionophore stimulated the release of β-hexosaminidase from mast cells. This effect was inhibited by compound **5** at the concentration of 40, 80, 160, and 320 μM. These effects were not due to cytotoxic effects. Compound **9** did not inhibit calcium ionophore A23187-induced mast cell activation at any of the concentrations tested.

Regarding morphological and morphometric studies, mast cells were easily identified by the presence of cytoplasmic secretory granules, which stain metachromatically (Fig. 5). Mast cells from the basal group are dominated by tightly packed secretory granules (Fig. 5A). Disruption of the cell surface and degranulation may be seen in mast cells stimulated with compound 48/80 (Fig. 5B) and with the calcium ionophore A23187 (Fig. 5E). The morphology of the cells treated with compound **5** (Fig. 5C and Fig. 5F, compound **5**+48/80 and compound **5**+A23187, respectively) or compound **9** (Fig. 5D and Fig. 5G, compound **9**+48/80 and compound **9**+A23187, respectively) shows a lower degree of degranulation than that of secretagogue samples. The number of degranulated mast cells increased compared to the basal group after incubation with 10 μg/ml compound 48/80 and with 0.12 μM calcium ionophore A23187 (Fig. 6). Preincubation of mast cells with compound **5** or with compound **9** inhibited the increase in the number of degranulated mast cells induced by 10 μg/ml compound 48/80 (Fig. 6). Preincubation of mast cells with compound **5** inhibited the increase of degranulated mast cells induced by 0.12 μM calcium ionophore A23187 (Fig. 6).

Fig. 7 shows mast cells observed under a transmission electron microscope. A representative view of the basal mast cell population is shown in Fig. 7A. These cells were characterized by a non-segmented nucleus with only moderate nuclear chromatin condensation, narrow surface folds, and numerous secretory granules that were regularly distributed throughout the cytoplasm. Most granules exhibited either round or oval

profiles and appeared homogeneously dense. A representative mast cell from the compound 48/80-treated group is shown in Fig. 7B. This cell showed obvious morphological changes and evidence of enhanced granule release by exocytosis compared to basal cells. Compound 48/80-stimulated mast cells showed an irregular nucleus and a cytoplasm with irregular secretory granules exhibiting different degrees of electron densities. Some granules exhibited a decreased electron density when compared to non-stimulated cells. The granular population appeared reduced compared to the basal group, and the granules were more separated. Perigranular electron-lucent spaces surrounded some secretory granules. Some exocytic cavities and intracytoplasmic degranulating channels could be seen. Figs. 7C and 7D show mast cells treated with compound **5** and compound **9**, respectively, for 10 min prior to the challenge with 10 μg/ml compound 48/80. These cells showed minimal degranulation, especially those cells treated with compound **9**. The granules' morphological characteristics and cytoplasmic distribution were similar to basal cells. Narrow perigranular spaces surrounded some granules. Fig. 7E shows a representative mast cell from the 0.12 μg/ml calcium ionophore A23187 group. The morphological characteristics of this cell are compatible with mast cell activation, although they are not as pronounced as those of the compound 48/80 group. Figs. 7F and 7G show mast cells treated with compound **5** and compound **9**, respectively, for 10 min prior to the challenge with 0.12 μg/ml calcium ionophore A23187. These cells showed minimal degranulation, especially those cells treated with compound **5**.

None of the β-substituted-α-halomethyl acrylate tested induced *per se* the release of β-hexosaminidase release from mast cells (data not shown).

We next performed a comparative efficacy study with sodium chromoglycate (chromolyn), a reference mast cell stabilizer. The inhibitory effects of compounds **5** and

9 were higher than those obtained with the reference compound sodium chromoglycate when mast cells were activated with compound 48/80. The inhibitory effect of compound **5** was higher than that obtained with the reference compound sodium chromoglycate when mast cells were activated with the calcium ionophore A23187. The values of IC50 for each compound are shown in Table 2.

Discussion

The Horner Wadsworth Emmons (HWE) reaction allows the generation of new carbon-carbon bonds from stabilized aldehydes or ketones and phosphonates. Based on the above reaction and series of substituted aromatic aldehydes, a new aqueous phase methodology was developed to obtain halogenated alkenes in the *alpha* position and aromatic substituents in the *beta* position. More specifically, from the current work, we have synthesized a series of eleven 3-substituted-2-bromo-methyl acrylates and 3-substituted-2-iode-methyl acrylates according to the synthetic route shown in Fig. 1. IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry fully characterized the structure of the products.

These structures have an interesting similarity with regard to methyl cinnamate, which is an organic compound that is found naturally in plants. It is used in the pharmaceutical and food industry. It has antimicrobial action and inhibitory effect on adipogenesis (Ernawati and Khoirunni'mah, 2015). Moreover, cinnamon extract inhibits the degranulation of inflammatory mediators in mast cells (Hagenlocher et al., 2013). Its structure is similar to β-substituted methyl α-haloacrylates. They have a halogen in the alpha position and a β-substituted aromatic group. Cinnamates, acrylates, α,β-unsaturated carbonyl compounds can act as Michael acceptors due to their structure (Cozzi, 2003). These compounds have various properties, including antioxidant activity, pro- and anti-

inflammatory activity, antiulcerogenic, antipyretic, antimicrobial, antidiabetic, and antitumor activity (Murakami et al., 2018).

It was possible to obtain a series of olefinic derivatives for the biological assays through a methodology that is simple to carry out in the laboratory and uses water as the HWE reaction medium. The new synthetic compounds obtained from the current work represent an extension of the previously synthesized variety of cinnamic acid derivatives that possess the carbonyl group of an α,β-unsaturated methyl ester, linked via the β carbon to an aromatic ring and linked via the α carbon to a halogen. These structures with α,β-unsaturated carbonyl groups are also part of synthetic structures with antitumor activity (Gelderblom et al., 2014). We propose that an intracellular nucleophile could carry out a first Michael attack step on the β carbon of the double bond, followed by an elimination reaction or a second nucleophilic substitution (Guirouilh-Barbat et al., 2009).

The aldehydes used in organic synthesis have an aromatic ring substituted with a group ($-NO_2$) or strongly electronegative atoms (F, Cl, Br), electronically deactivating the carbonyl carbon. This characteristic, together with the own inductive effect of carbonyl oxygen on the double-bonding electrons, favored the nucleophilic attack by the phosphonate carbanion on the carbonyl carbon of the aldehyde during the HWE reaction. Like other authors (Jafari and Ghadami, 2016), the yields of trisubstituted alkenes were notably higher (>90%) when p-nitro benzaldehyde was used as a precursor reagent (compounds 1 and 2). When p-chlorobenzaldehyde was used, the yield was also >90%, while using p-fluorobenzaldehyde, the yield was almost 90%. In both cases, both stereoisomers were purified. We attribute these results to the aforementioned electronic effect of the chloro group in the *para* position on the aromatic ring, with reaction times of approximately 2 h, similar to those obtained by Jafari and Ghadami (2016), considering that both methodologies took place in an aqueous medium.

The HWE reaction is an efficient tool for obtaining carbon-carbon bonds, giving α,β-unsaturated products with high stereoselectivity, in which the largest groups are on opposite sides of the double bond (Xiao et al., 2021). In our methodology, by introducing a halogen in the alpha position, we can obtain both isomers, *Z* and *E*. The trans and cis products are defined by the priority assignment of the substituent groups to the double bond according to the rules of Cahn Ingold and Prelog (Mata and Lobo, 2005). The largest group corresponds to GAE, in this case, the methyl ester. The most sterically hindered was the least polar product (*E*) and, in all cases, the first to be purified by column. The least sterically hindered turned out to be the most polar (*Z*), the one retained for the longest time in the silica gel column.

After having succeeded in synthesizing the 2-bromo-3-substituted methyl acrylates and 2-iode-3-substituted methyl acrylates, our next objective was to analyze the effect of each of these 11 new compounds in an *in vitro* biological and pharmacological model of mast cell activation. This decision was made considering the critical role played by mast cells in the pathogenesis of immune, inflammatory, and tumor diseases (Yang et al., 2023).

In order to carry out this second part of the work, purified mast cells were preincubated with increasing concentrations of each of the newly synthesized for 10 min and stimulated with 10 μg/ml compound 48/80 for another 10 min at 37ºC. The enzyme β-hexosaminidase, a mediator preformed and stored in mast cell granules, was then measured as a marker of mast cell activation and degranulation (Sahid and Kiyoi, 2020). As expected, the secretagogue was effective in inducing mast cell degranulation. This effect was inhibited by compound **5** at the concentration of 320 μM and by compound **9** at 160 and 320 μM without causing cytotoxic effects. Compound 48/80 is used as a classical mast cell activator for IgE-independent G proteins and is the most commonly used activation method in pseudo-allergy studies (Zhang et al., 2022). This basic

secretagogue activates rat Mas-related-GPCR-B3 receptor (MRGPRB3), mouse MRGPRB2 and human MRGPRX2, which are present in mast cells (Sahid et al., 2020). Muscle relaxants, antibiotics, and several neuropeptides have been shown to activate mast cells through these receptors and mediate drug pseudo-allergic reactions in animal models (Sahid et al., 2020).

At this point, we wanted to analyze the effect of compounds **5** and **9** on mast cell activation induced by a secretagogue acting by mechanisms other than compound 48/80. So, the action of these new molecules on mast cell degranulation induced by the calcium ionophore A23187 was studied. This ionophore stimulates mast cells through intracellular calcium increase, that is, by a common point in which both IgE-dependent and IgE-independent manners coincide. As expected, the calcium ionophore effectively induced mast cell degranulation in our study. This effect was inhibited by compound **5** at 40, 80, 160, and 320 μM concentrations without affecting cell viability. Compound **9** did not inhibit calcium ionophore A23187-induced mast cell activation at any of the concentrations tested. These results suggest that the inhibitory action of compound **5** may be downstream of cytosolic calcium increase, whereas the mechanism of compound **9** may be downstream of MRGPR activation.

Our morphological findings, at the light and electron microscope level, reinforced the validity of our results. Microscopy studies are consistent with the β-hexosaminidase release findings, showing the interaction of compounds **5** and **9** with the mast cell population and inhibition of the degranulation induced by the secretagogues.

Additionally, we demonstrated that the inhibitory effects exhibited by compounds **5** and **9** were more potent than that of the reference compound, sodium chromoglycate, when mast cells were stimulated by compound 48/80. We also demonstrated that the inhibitory effect exhibited by compound **5** was more potent than sodium chromoglycate's when the

calcium ionophore A23187 stimulated mast cells. These results could be explained based on the different chemical structures and steric hindrances for nucleophilic attacks of the β-substituted-α-halomethyl acrylates and the different nature of the mast cell targets. If an analysis of all the molecular structures that characterize the synthetic compounds is made, it can be observed that the substructure of each one presents a conjugated bond to a carbonyl group of an ester, which makes possible a Michael addition. However, the variations introduced in each reaction were small from the structural point of view but important enough to make one compound different from another from the point of view of its electronic environment and spatial arrangement. This comment means that the interaction of each molecule is not the same at the cellular or subcellular level compared to another. Several speculations could be made about this, but it would be more prudent to carry out future studies to elucidate the mechanisms of action of these compounds at the cellular and molecular levels.

It is essential to highlight the novelty of the reaction methodology used in this work. Moreover, it is unusual for a single work to present the proposal of a complete series of new synthetic molecules and, in addition, to explore their biological activity. From this crucial point, the compounds that demonstrated significant bioactivity have become leading compounds that open all the doors of organic synthesis through which it is possible to carry out new structural modifications.

Conclusions

In conclusion, this is the first report about the synthesis “de novo” of a series of 3-substituted-2-bromo methyl acrylates and 3-substituted-2-iode methyl acrylates through a modified Horner-Wadsworth-Emmons (HWE) reaction in an aqueous medium. Using an aqueous phase enables environmental care and leads to high cost-effectiveness and

waste reduction. This simple, controllable, and economical method would potentially apply HWE reaction in the chemical industry.

On the other hand, this is the first time it has been reported that the new synthetic drugs methyl (*Z*)-2-bromo-3-(furan-3-yl)acrylate and methyl (*E*)-2-bromo-3-(3-bromophenyl)acrylate strongly inhibit mast cell degranulation, by decreasing release of preformed β-hexosaminidase, without affecting cell viability. The implications of these results are relevant as a basis for developing new anti-inflammatory and mast cell stabilizing drugs.

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Conflict of Interest

The authors declare no conflict of interest.

References

Arthur, G., Bradding, P., 2016. New Developments in Mast Cell Biology: Clinical Implications. Chest 150, 680–693. https://doi.org/10.1016/j.chest.2016.06.009

Berdún, S., Rychter, J., Vergara, P., 2015. Effects of nerve growth factor antagonist K252a on peritoneal mast cell degranulation: Implications for rat postoperative ileus. Am J Physiol Gastrointest Liver Physiol 309, 801–806. https://doi.org/10.1152/ajpgi.00152.2015

Bulfone-Paus, S., Nilsson, G., Draber, P., Blank, U., Levi-Schaffer, F., 2017. Positive and Negative Signals in Mast Cell Activation. Trends Immunol 38, 657–667. https://doi.org/10.1016/j.it.2017.01.008

Ceñal, J.P., Carreras, C.R., Tonn, C.E., Padrón, J.I., Ramírez, M.A., Díaz, D.D., García-Tellado, F., Martín, V.S., 2005. Acid-mediated highly regioselective oxidation of substituted furans: A simple and direct entry to substituted butenolides. Synlett 1575–1578. https://doi.org/10.1055/s-2005-869865

Coll, R.C., Vargas, P.M., Mariani, M.L., Penissi, A.B., 2020. Natural α,β-unsaturated lactones inhibit neuropeptide-induced mast cell activation in an in vitro model of neurogenic inflammation. Inflammation Research 69, 1039–1051. https://doi.org/10.1007/s00011-020-01380-8

Cozzi, P., 2003. The discovery of a new potential anticancer drug: A case history. Farmaco 58, 213–220. https://doi.org/10.1016/S0014-827X(03)00014-4

Ernawati, T., Khoirunni'mah, Z., 2015. 70 SYNTHESIS METHYL NITROPHENYL ACRYLATE AND CYTOTOXIC ACTIVITY TEST AGAINST P388 LEUKEMIA CELLS, Indones. J. Chem.

Ettari, R., Previti, S., Cosconati, S., Maiorana, S., Schirmeister, T., Grasso, S., Zappalà, M., 2016. Development of novel 1,4-benzodiazepine-based Michael acceptors as antitrypanosomal agents. Bioorg Med Chem Lett 26, 3453–3456. https://doi.org/10.1016/j.bmcl.2016.06.047

Falduto, G.H., Pfeiffer, A., Luker, A., Metcalfe, D.D., Olivera, A., 2021. Emerging mechanisms contributing to mast cell-mediated pathophysiology with therapeutic implications. Pharmacol Ther 220, 107718. https://doi.org/10.1016/j.pharmthera.2020.107718

Frossi, B., Mion, F., Sibilano, R., Danelli, L., Pucillo, C.E.M., 2018. Is it time for a new classification of mast cells? What do we know about mast cell heterogeneity? Immunol Rev 282, 35–46. https://doi.org/10.1111/imr.12636

Gehringer, M., Laufer, S.A., 2019. Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology. J Med Chem 62, 5673–5724. https://doi.org/10.1021/acs.jmedchem.8b01153

Gelderblom, H., Blay, J.Y., Seddon, B.M., Leahy, M., Ray-Coquard, I., Sleijfer, S., Kerst, J.M., Rutkowski, P., Bauer, S., Ouali, M., Marreaud, S., Van Der Straaten, R.J.H.M., Guchelaar, H.J., Weitman, S.D., Hogendoorn, P.C.W., Hohenberger, P., 2014. Brostallicin versus doxorubicin as first-line chemotherapy in patients with advanced or metastatic soft tissue sarcoma: An European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group randomised phase II and pharmacogenetic study. Eur J Cancer 50, 388–396. https://doi.org/10.1016/j.ejca.2013.10.002

Giannetti, A., Filice, E., Caffarelli, C., Ricci, G., Pession, A., 2021. Mast cell activation disorders. Medicina (Lithuania) 57, 1–13. https://doi.org/10.3390/medicina57020124

Gu, X., Jiang, Y., Chen, J., Zhang, Y., Guan, M., Li, X., Zhou, Q., Lu, Q., Qiu, J., Yin, X., 2019. Synthesis and biological evaluation of bifendate derivatives bearing acrylamide moiety as novel antioxidant agents. Eur J Med Chem 162, 59–69. https://doi.org/10.1016/j.ejmech.2018.11.003

Guirouilh-Barbat, J., Zhang, Y.W., Pommier, Y., 2009. Induction of glutathione-dependent DNA double-strand breaks by the novel anticancer drug brostallicin. Mol Cancer Ther 8, 1985–1994. https://doi.org/10.1158/1535-7163.MCT-09-0320

Hagenlocher, Y., Bergheim, I., Zacheja, S., Schäffer, M., Bischoff, S.C., Lorentz, A., 2013. Cinnamon extract inhibits degranulation and de novo synthesis of inflammatory mediators in mast cells. Allergy: European Journal of Allergy and Clinical Immunology 68, 490–497. https://doi.org/10.1111/all.12122

Inagaki, N., Nakai, N., Kimata, M., Kawasaki, H., Nagai, H., 2001. Recovery of purification-associated reduction in antigen-induced histamine release from rat peritoneal mast cells. Biol Pharm Bull 24, 829–834. https://doi.org/10.1248/bpb.24.829

Jackson, P.A., Widen, J.C., Harki, D.A., Brummond, K.M., 2017. Covalent Modifiers: A Chemical Perspective on the Reactivity of α,β-Unsaturated Carbonyls with Thiols via Hetero-Michael Addition Reactions. J Med Chem 60, 839–885. https://doi.org/10.1021/acs.jmedchem.6b00788

Jafari, A.A., Ghadami, M., 2016. Efficient synthesis of α,β-unsaturated ketones with trans-selective Horner–Wadsworth–Emmons reaction in water. Environ Chem Lett 14, 223–228. https://doi.org/10.1007/s10311-016-0552-8

Lukan, N., 2020. “Cytokine storm”, not only in COVID-19 patients. Mini-review. Immunol Lett 228, 38–44. https://doi.org/10.1016/j.imlet.2020.09.007

Magalhaes, L.G., Ferreira, L.L.G., Andricopulo, A.D., 2018. Recent advances and perspectives in cancer drug design. An Acad Bras Cienc 90, 1233–1250. https://doi.org/10.1590/0001-3765201820170823

Mata, P., Lobo, A.M., 2005. The Cahn, Ingold and Prelog System: Eliminating ambiguity in the comparison of diastereomorphic and enantiomorphic ligands.

Tetrahedron Asymmetry 16, 2215–2223. https://doi.org/10.1016/j.tetasy.2005.05.037

Monticelli, S., Leoni, C., 2017. Epigenetic and transcriptional control of mast cell responses. F1000Res 6, 1–11. https://doi.org/10.12688/f1000research.12384.1

Murakami, Y., Kawata, A., Suzuki, S., Fujisawa, S., 2018. Cytotoxicity and pro-/anti-inflammatory properties of cinnamates, acrylates and methacrylates against RAW264.7 cells. In Vivo (Brooklyn) 32, 1309–1321. https://doi.org/10.21873/invivo.11381

Penissi, A.B., Vera, M.E., Mariani, M.L., Rudolph, M.I., Ceñal, J.P., de Rosas, J.C., Fogal, T.H., Tonn, C.E., Favier, L.S., Giordano, O.S., Piezzi, R.S., 2009. Novel anti-ulcer α,β-unsaturated lactones inhibit compound 48/80-induced mast cell degranulation. Eur J Pharmacol 612, 122–130. https://doi.org/10.1016/j.ejphar.2009.03.052

Persia, F.A., Mariani, M.L., Fogal, T.H., Penissi, A.B., 2014. Hydroxytyrosol and oleuropein of olive oil inhibit mast cell degranulation induced by immune and non-immune pathways. Phytomedicine 21, 1–6. https://doi.org/10.1016/j.phymed.2014.05.010

Puri, N., Roche, P.A., 2008. Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. Proc Natl Acad Sci U S A 105, 2580–2585. https://doi.org/10.1073/pnas.0707854105

Romagnoli, R., Baraldi, P.G., Cruz-Lopez, O., Cara, C.L., Carrion, M.D., Balzarini, J., Hamel, E., Basso, G., Bortolozzi, R., Viola, G., 2010. Symmetrical α-bromoacryloylamido diaryldienone derivatives as a novel series of antiproliferative agents. Design, synthesis and biological evaluation. Bioorg Med Chem Lett 20, 2733–2739. https://doi.org/10.1016/j.bmcl.2010.03.075

Romagnoli, R., Baraldi, P.G., Prencipe, F., Oliva, P., Baraldi, S., Salvador, M.K., Lopez-Cara, L.C., Bortolozzi, R., Mattiuzzo, E., Basso, G., Viola, G., 2017. Design, synthesis and biological evaluation of 3-substituted-2-oxindole hybrid derivatives as novel anticancer agents. Eur J Med Chem 134, 258–270. https://doi.org/10.1016/j.ejmech.2017.03.089

Roman, D., Sauer, M., Beemelmanns, C., 2021. Applications of the Horner-Wadsworth-Emmons Olefination in Modern Natural Product Synthesis. Synthesis (Germany) 53, 2713–2739. https://doi.org/10.1055/a-1493-6331

Sahid, M.N.A., Kiyoi, T., 2020. Mast cell activation markers for in vitro study. J Immunoassay Immunochem 41, 778–816. https://doi.org/10.1080/15321819.2020.1769129

Sahid, M.N.A., Liu, S., Mogi, M., Maeyama, K., 2020. Tachykinin-1 receptor antagonism suppresses substance-P- and compound 48/80-induced mast cell activation from rat mast cells expressing functional mas-related GPCR B3. Inflammation Research 69, 289–298. https://doi.org/10.1007/s00011-020-01319-z

Song, J.L., Zhang, J., Liu, C.L., Liu, C., Zhu, K.K., Yang, F.F., Liu, X.G., Figueiró Longo, J.P., Alexandre Muehlmann, L., Azevedo, R.B., Zhang, Y.Y., Guo, Y.W., Jiang, C.S., Zhang, H., 2017. Design and synthesis of pregnenolone/2-cyanoacryloyl conjugates with dual NF-κB inhibitory and anti-proliferative activities. Bioorg Med Chem Lett 27, 4682–4686. https://doi.org/10.1016/j.bmcl.2017.09.013

Varricchi, G., De Paulis, A., Marone, G., Galli, S.J., 2019. Future needs in mast cell biology. Int J Mol Sci 20, 1–25. https://doi.org/10.3390/ijms20184397

Vera, M.E., Mariani, M.L., Aguilera, C., Penissi, A.B., 2021. Effect of a cytoprotective dose of dehydroleucodine, xanthatin, and 3-benzyloxymethyl-5h-furan-2-one on

gastric mucosal lesions induced by mast cell activation. Int J Mol Sci 22. https://doi.org/10.3390/ijms22115983

Vera, M.E., Persia, F. a., Mariani, M.L., Rudolph, M.I., Fogal, T.H., Ceñal, J.P., Favier, L.S., Tonn, C.E., Penissi, A.B., 2012. Activation of human leukemic mast cell line LAD2 is modulated by dehydroleucodine and xanthatin. Leuk Lymphoma 53, 1795–1803. https://doi.org/10.3109/10428194.2012.662644

Xiao, H.X., Yan, Q.X., He, Z.H., Zou, Z.B., Le, Q.Q., Chen, T.T., Cai, B., Yang, X.W., Luo, S.L., 2021. Total synthesis and anti-inflammatory bioactivity of (−)-majusculoic acid and its derivatives. Mar Drugs 19. https://doi.org/10.3390/md19060288

Yang, B.-G., Kim, A.-R., Lee, D., An, S.B., Shim, Y.A., Jang, M.H., 2023. Degranulation of Mast Cells as a Target for Drug Development. Cells 12, 1506. https://doi.org/10.3390/cells12111506

Yeung, Y.T., Aziz, F., Guerrero-Castilla, A., Arguelles, S., 2018. Signaling Pathways in Inflammation and Anti-inflammatory Therapies, Current Pharmaceutical Design. https://doi.org/10.2174/1381612824666180327165604

Zhang, P., Wang, Y., Zhang, J., Hong, T., 2022. Allantoin Inhibits Compound 48/80-Induced Pseudoallergic Reactions In Vitro and In Vivo. Molecules 27. https://doi.org/10.3390/molecules27113473

Zhang, T., Finn, D.F., Barlow, J.W., Walsh, J.J., 2016. Mast cell stabilisers. Eur J Pharmacol 778, 158–168. https://doi.org/10.1016/j.ejphar.2015.05.071

Figure legends

Fig. 1.

Modified Horner-Wadsworth-Emmons (HWE) reaction.

Fig. 2.

Effect of varying concentrations of 11 β-substituted-α-halo- methyl acrylates on mast cell β-hexosaminidase release induced by 10 μg/ml compound 48/80. Results are expressed as the percentage of β-hexosaminidase release. Values are presented as means ± S.E.M. *P<0.05, *”P<0.01 and ***P<0.001.

Fig. 3.

Effect of varying concentrations of 11 β-substituted-α-halo- methyl acrylates and compound 48/80 (10 μg/ml) on the percentage of mast cell viability. Results are expressed as the percentage of cell viability. Values are presented as means ± S.E.M. *P<0.05, *”P<0.01 and ***P<0.001.

Fig. 4.

Effect of varying concentrations of compound **5** and compound **9** on mast cell β-hexosaminidase release induced by the calcium ionophore A23187 (0.12 μg/ml), and on the percentage of mast cell viability and as the percentage of cell viability. Values are presented as means ± S.E.M. *P<0.05, **P<0.01 and ***P<0.001.

Fig. 5.

Light-microscopic photographs of rat peritoneal mast cells stained with toluidine blue. Purified mast cells were preincubated with 320 μM compound **5** or 320 μM compound **9** for 10 min and stimulated with 10 μg/ml compound 48/80 or 0.12 μg/ml A23187 for another 10 min at 37ºC. After incubations, cells were fixed for light microscopy. Fig. 5A: basal; Fig. 5B: compound 48/80; Fig. 5C: compound **5** + 48/80; Fig. 5D: compound **9** +

48/80; Fig. 5E: A23187; Fig. 5F: compound **5** + A23187; Fig. 5G: compound **9** + A23187. Scale bar = 50 μm.

Fig. 6.

Effect of 320 μM compound **5**, or 320 μM compound **9** on mast cell degranulation induced by 10 μg/ml compound 48/80 or 0.12 μg/ml A23187. Results are expressed as the percentage of degranulated mast cells. Values are presented as means ± SEM. *P<0.05 and *P<0.01 among groups.

Fig. 7.

Representative transmission electron micrographs showing rat peritoneal mast cells. **A**: basal; **B**: 10 μg/ml compound 48/80; **C**: 320 μM compound **5** + 48/80: **D**: 320 μM compound **9** + 48/80; **E**: 0.12 μg/ml calcium ionophore A23187; **F**: 320 μM compound **5** + A23187; **G**: 320 μM compound **9** + A23187. Scale bar: 2 μm.

Table legends

Table 1

Relevant compounds synthesized by the modified Horner-Wadsworth-Emmons (HWE) reaction.

Table 2

IC50 (μM) ± SEM values for inhibitory activity of compound 5, compound 9, and sodium chromoglycate on compound 48/80- and the calcium ionophore A23187-induced mast cell activation.