Comparative analysis of the *in vitro* cytotoxicity of the dietary biogenic
amines tyramine and histamine

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Abstract

Tyramine and histamine, the most toxic biogenic amines (BA), are often found in high concentrations in certain foods. Prompted by the limited knowledge of BA toxicity, and increasing awareness of the risks associated with high intakes of dietary BA, the *in vitro* cytotoxicity of tyramine and histamine was investigated. Tyramine and histamine were toxic for HT29 intestinal cell cultures at concentrations commonly found in BA-rich food, as determined by real-time cell analysis. Surprisingly, tyramine had a stronger and more rapid cytotoxic effect than histamine. Their mode of action was also different, while tyramine caused cell necrosis, histamine induced apoptosis. To avoid health risks, the BA content of foods should be reduced and legal limits established for tyramine.

Keywords: Biogenic amines; Cytotoxicity; Food security; Histamine; Tyramine.

Chemical compounds studied in this article

Tyramine (PubChem CID: 66449); Histamine (PubChem CID: 5818)

1. Introduction

Biogenic amines (BA) are described as biologically active, low molecular weight organic bases that may accumulate in some foods owing to the undesirable microbial decarboxylation of certain amino acids (Linares, Martin, Ladero, Alvarez, & Fernandez, 2011; Naila, Flint, Fletcher, Bremer, & Meerdink, 2010). BA-producing microorganisms may be contaminants, but may also belong to groups that participate in food fermentation. In fact, certain bacterial species widely used in the dairy industry, and which enjoy 'generally regarded as safe' (GRAS) status, have been typified as BA producers (Fernandez, Linares, & Alvarez, 2004; Ladero, Rattray, Mayo, Martin, Fernandez, & Alvarez, 2011; Linares, del Rio, Ladero, Redruello, Martin, Fernandez, et al., 2013).

Foods likely to contain high BA concentrations are fish and fish products, along with fermented foods and beverages such as cheese and wine (European Food Safety Authority, 2011; Linares, del Rio, Ladero, Martinez, Fernandez, Martin, et al., 2012). The presence of BA in food is deemed a biological hazard by the Food and Agriculture Organization of the United Nations (FAO) (Food and Agriculture Organization of the United Nations, 2014). Although cheese has been implicated in outbreaks of histamine poisoning, such incidents have long been associated with the ingestion of spoiled fish of the *Scombroidae* family (which gave rise to the term 'scombroid syndrome') (Food and Drug Administration, 2001; Visciano, Schirone, Tofalo, & Suzzi, 2014). Typically, such poisoning is manifested as

allergic reactions, acute gastrointestinal distress, and perhaps neurological and cutaneous symptoms (Eastaugh & Shepherd, 1989). The term 'cheese reaction' was coined to refer to the symptoms - hypertension, migraine, headaches and other neurological problems - induced following the ingestion of cheese with high tyramine concentrations (Price & Smith, 1971; Shalaby, 1996; Stratton, Hutkins, & Taylor, 1991). These problems can be particularly severe in people who are susceptible owing to a reduced capacity to detoxify BA in the gut (Ladero, Calles-Enriquez, Fernandez, & Alvarez, 2010; Spano, Russo, Lonvaud-Funel, Lucas, Alexandre, Grandvalet, et al., 2010). This capacity may vary due to genetic reasons, but risk factors such as gastrointestinal disease, the taking of certain medications and the intake of alcohol can reduce the activity of the detoxifying enzymes (Ladero, Calles-Enriquez, Fernandez, & Alvarez, 2010).

Although it is generally accepted that BAs should not be allowed to accumulate in food products, legislation in this respect is insufficient. It is very difficult to establish a uniform maximum limit for ingested BAs since their toxic effect depends on the type of BA in question, the presence of modulating compounds, and on the efficiency of each person's detoxification system. The only BA for which maximum limits have been legally set by the European Food Safety Authority (EFSA) is histamine, and then only in scombroid-like fish (200 mg/kg) and fish products (400 mg/kg) (European Commission, 2005). The US Food and Drug Administration (FDA) suggested histamine concentrations in fish of over 500 mg/kg to be a danger to health

(Food and Drug Administration, 1996, 2001). No further legislation exists anywhere to regulate histamine or the remaining BAs in any other food.

Recently, the EFSA Panel on Biological Hazards (BIOHAZ) conducted a qualitative risk assessment for BAs in fermented foods, and concluded that our present knowledge of their toxicity was limited, and that further research was needed (European Food Safety Authority, 2011). Using real-time cell analysis (RTCA), the present work examines the *in vitro* toxicity of tyramine and histamine (the predominant BA in food) on human intestinal cells. The gastrointestinal mucosa, which is composed mainly of a monolayer of intestinal epithelial cells (IEC), represents the first point of contact with orally ingested BA. Cells belonging to the HT29 intestinal cell line, used as an *in vitro* model of IEC, were therefore exposed to different doses of tyramine and histamine and their cytotoxicity, including their capacity to induce necrosis and apoptosis, determined. The IC_{50} , the 'non-observed adverse effect level' (NOAEL) and the 'lowest observed adverse effect level' (LOAEL) values were calculated for each BA.

2. Material and Methods

2.1. Cell line and growth conditions

The intestinal cell line HT29 (ECACC 91072201), derived from a human colorectal adenocarcinoma, was purchased from the European Collection of Cell Cultures and used to create an *in vitro* model of the intestinal epithelium.

The HT29 cells were routinely cultured in McCoy's 5a medium supplemented with 10% heat-inactivated foetal bovine serum plus a mixture of antibiotics (50 µg/ml penicillin, 50 µg/ml streptomycin, 50 µg/ml gentamicin and 1.25 µg/ml amphotericin B). All media and reagents were purchased from Sigma-Aldrich (Madrid, Spain). All manipulations required for culturing, passaging (144 to 149 passages were performed) and maintenance of the cell line were undertaken in a 5% CO₂ atmosphere at 37°C, in a SL Waterjacked CO₂ Incubator (Sheldon Mfg. Inc., Cornelius, OR, USA), following standard procedures (Ruas-Madiedo, Medrano, Salazar, De Los Reyes-Gavilan, Perez, & Abraham, 2010).

2.2. Real-time cell analysis

The RTCA system is used to gather information on cell proliferation, migration and cytotoxicity via changes in cell morphology and adhesion (Atienzar, Tilmant, Gerets, Toussaint, Speeckaert, Hanon, et al., 2011). An xCelligence Real-Time Cell Analyzer (ACEA Bioscience Inc., Roche Applied Science, Germany) was used as previously described (Limame, Wouters, Pauwels, Fransen, Peeters, Lardon, et al., 2012) to detect any changes in the proliferation, migration adhesion or morphology of the HT29 intestinal cells following their treatment with different doses of tyramine [4-(2-aminoethyl)phenol hydrochloride] (Acros Organics, Belgium) and histamine [2-(4-Imidazolyl)ethylamine dihydrochloride] (Sigma-Aldrich). Cells were seeded in 16-well E-Plates (Roche Applied Science) equipped with gold microelectrode sensors at the bottom. These sensors generate an electric

field when a low voltage (<20 mV) is applied between the electrodes. Real-time measurements of the electrical impedance (referred to as the cell index) across the interdigitated microelectrodes allows for dynamic monitoring.

Briefly, HT29 cells were seeded at a density of 2×10^4 cells/well (for studies on proliferating cells) or 2×10^5 cells/well (for studies on confluent cells) in 16-well E-Plates containing 100 μ l of medium per well, and then incubated and monitored in a Heracell-240 Incubator (Thermo Electron LDD GmbH, Langenselbold, Germany) at 37°C and with a 5% CO₂ atmosphere (Hidalgo-Cantabrana, Kekkonen, de los Reyes-Gavilan, Salminen, Korpela, Gueimonde, et al., 2014). Stock solutions of tyramine and histamine were dissolved in water and adjusted to pH 6.8. Approximately 20 h after seeding, the cells were treated with one of 13 concentrations of tyramine (0 to 28.9 mM) or histamine (0 to 129.7 mM). The cell index was monitored for 24 h for proliferation studies, and for 30 h for confluence studies, under the same conditions. The cell index was normalized to the time point just before the addition of the BA and set to 1. For each condition, measurements were performed in triplicate.

Dose-response curves for tyramine and histamine were constructed using RTCA software, plotting the normalized cell index at 24 h of treatment against the log₁₀ value of the corresponding BA concentration. Non-linear regression trend lines were fitted to sigmoid dose-response (variable slope) curves using SigmaPlot 13.0 software (Systat Software Inc., San Jose, CA, USA). This software also provided the coefficient of determination (R^2) as a

measurement of the goodness of fit of the data to the curves, as well as the Hill slope value, which indicates the steepness of the curve.

The IC₅₀ was defined as the concentration of BA required to achieve half of the strongest cytotoxic effect observed by RTCA. IC₅₀ values for tyramine and histamine were calculated in proliferating cultures using RTCA software to construct a sigmoid dose response curve for arbitrary time points (8 h, 12 h, 18 h and 24 h) of BA exposure.

2.3. Fluorescence microscopy: nuclear DNA staining

Cells were seeded at a density of 2×10^5 cells/well, and incubated in flat-bottom microplates under identical conditions to those used in the RTCA studies. After 20 h of incubation the cells were treated with one of six different concentrations of tyramine (0 to 17.3 mM) or histamine (0 to 21.7 mM). At 24 h post-treatment, the cells were washed with 0.1 M PBS pH 7.2, fixed with 3.7% paraformaldehyde for 15 min, permeabilised by immersion in absolute ethanol for 5 min, and then labelled with 4'6-diamino-2-phenylindole dihydrochloride (DAPI) (Thermo Fisher Scientific Inc., Madrid, Spain) by incubation for 5 min with the stain (DAPI 300 nM in 0.1 M PBS pH 7.2). Once the labelling solution was aspirated, the cells were rinsed three times in PBS, and each well of the microplate was viewed using a 3-colour LumaScope-600 epifluorescence microscope (Etaluma Inc., CA, USA).

2.4. Cell apoptosis

One of the most established hallmarks of apoptosis is DNA fragmentation (Saraste & Pulkki, 2000). The commercially available Cellular DNA Fragmentation ELISA Kit (Roche Applied Science) was used to measure apoptosis-associated DNA fragments in the cytoplasm. This assay is based on the quantitative “sandwich enzyme immunoassay” (ELISA) principle, and involves two mouse monoclonal antibodies directed against DNA and 5'-bromo-2'-deoxyuridine (BrdU). Briefly, HT29 cells were seeded at 2×10^4 cells/well in 96-well plates and incubated with BrdU for 20 h at 37°C in a 5% CO₂ atmosphere. After this time, proliferating cells were exposed to the corresponding BA dose for 24 h. In parallel with the BA-treated samples, a negative control (untreated cells) was established to determine the spontaneous release of DNA fragments, and a positive control to estimate the incorporation of BrdU into the genomic DNA (cells treated with 1% Triton-X100). Cells were collected after centrifugation at 250 g for 10 min and lysed with the incubation solution for 30 min at 25°C. After further centrifugation at 250 g for 10 min, the resulting supernatant was transferred to a microplate precoated with anti-DNA antibodies, followed by a standard ELISA assay to detect the BrdU-labelled DNA fragments. Finally, apoptosis-associated DNA was determined at 450 nm in a Benchmark Plus Microplate Spectrophotometer (Bio-Rad, Madrid, España). DNA fragmentation was expressed as the fold increase of the control values.

2.5. Cell lysis assay

RTCA cell culture supernatants collected after 24 h of incubation with the corresponding dose of tyramine or histamine were tested for the presence of lactate dehydrogenase activity (LDH) using the LDH Assay Kit (Roche Applied Science), following the manufacturer's instructions. The release of LDH into the medium is a marker of the cell membrane damage that occurs in necrosis. Negative (no lysing reagent) and positive controls (with lysis reagent) were run in parallel.

2.6. Data and statistical analysis

The results obtained in all experiments are expressed as the means $\pm$ standard deviation of three independent replicates. Statistical treatment involved ANOVA followed by Fisher's multiple comparison test; this was performed using SigmaPlot software. Significance was set at $p < 0.05$ (indicated in figures with an asterisk).

The NOAEL value was identified as the highest concentration of BA that caused no detectable adverse effect on the target cells. The LOAEL value was defined as the lowest concentration of BA that produced a detectable adverse effect.

3. Results

3.1. Dynamic cell responses of tyramine and histamine-treated intestinal cells

To determine whether tyramine and histamine treatments perturbed cell homeostasis and induced cytotoxic effects in the HT29 cells, dose-response curves were constructed in real time by the RTCA system. Approximately 20 h after seeding, different concentrations of each BA were added and the cells monitored for 24 h (Fig. 1). It was found that the concentration of histamine and tyramine remained the same after the 24 hours (data not shown), suggesting insignificant amino oxidase activities in HT29 cell cultures.

The curves showed that cells proliferated over the 20 h of culture before BA treatment. After this time, the curves for BA-untreated cultures showed continued proliferation. However, a drastic reduction in the normalized cell index was seen when the cells were exposed to increasing concentrations of tyramine (Fig. 1A) or histamine (Fig. 1B). Both BAs were associated with a dose-dependent cytotoxic effect. The effect of tyramine was very acute and rapid: the normalized cell index of proliferating cells treated with concentrations above 2.9 mM dropped within a few minutes. The cytotoxicity of the histamine was less severe than that of tyramine: cells treated with 5 mM histamine showed no change in their proliferation curves. However, doses of 10.8 mM or higher were cytotoxic, although more moderately and less acutely so than observed for cytotoxic doses of tyramine. The cytotoxic effects of both BAs on confluent HT29 cells (data not shown) were similar to those seen on proliferating cells.

Dose-response curves for tyramine and histamine were constructed 24 h after BA exposure (Fig. 2). Both that of tyramine (Fig. 2A) and histamine (Fig.

2B) fitted a sigmoid curve with a R^2 of 0.9823 and 0.9942 respectively. The Hill slope of the curve for tyramine (-3.14) was steeper than that for histamine (-1.44). Thus, small increases in tyramine concentration had a greater toxic effect than small increases in histamine.

The greater cytotoxicity of tyramine was confirmed when the IC_{50} values for each BA were compared at 8 h, 12 h, 18 h and 24 h (Table 1). For each time point, the RTCA data fitted a sigmoid dose-response curve with an R^2 of >0.995 in all cases. The IC_{50} values showed both BAs to have a dose-dependent cytotoxic effect on the HT29 cells (Table 1). Tyramine was approximately 10 times more toxic than histamine (IC_{50} of tyramine after 24 h of treatment 3.2 ± 0.04 mM, compared to 26.0 ± 1.2 mM for histamine). Thus, a 10-times lower concentration of tyramine than histamine is needed to achieve half of the latter's strongest cytotoxic effect. The results also indicated that the cytotoxic effects of tyramine did not increase with incubation times beyond 18 h. In contrast, sensitivity to histamine increased over time.

The NOAEL and LOAEL values for tyramine (Fig. 2A) were 1.8 mM and 2.2 mM respectively, and 3 mM and 4 mM respectively for histamine. Thus, the cells showed a higher toxicological threshold for histamine.

3.2. Fluorescence microscopy examination of tyramine- and histamine-treated cell cultures

To visualize cytotoxic effects, cell cultures treated for 24 h with a range of tyramine or histamine concentrations were stained with DAPI and observed by fluorescence microscopy (Fig. 3). The number of cells decreased inversely with the concentration of both BAs, although the tyramine had a more severe effect. Tyramine doses above 2.9 mM had a more serious effect on the cells, indeed, at the two highest concentrations tested (11.5 mM and 17.3 mM) very few cells were observed. The cytotoxicity of histamine began to become evident at higher concentrations (above 5 mM), but even beyond the maximum concentration of tyrosine used, the cytotoxicity of histamine was less severe.

The chromatin of the histamine-treated cells appeared more condensed - a characteristic of apoptosis - than the chromatin of the tyramine-treated cells (Fig. 3). This suggests that the mode of action of these BAs might be different. Further experiments to examine this were therefore performed.

3.3. Apoptosis assays

Intracellular DNA fragments typical of apoptotic cells were sought using the cellular DNA fragmentation assay. To ensure the meeting of the necessary cell death test conditions, endpoint times of 24 h were employed (the time needed by each BA to induced maximum cell damage). Intermediate doses - 5.8 mM for tyramine and 16.3 mM for histamine – were used to ensure that cells would be damaged though not all would be killed. After BA-treatment of the BrdU-labelled cells, DNA fragments were extracted from the cytoplasm

and quantified. DNA-fragmentation detected in these cells was slightly increased (12.3%) in comparison with controls. In contrast, DNA fragmentation in the cells exposed to histamine increased by 43.9% (Fig. 4). This strongly suggests that histamine, much more so than tyramine, exerts an apoptotic effect.

3.4. Assessment of cytolytic (necrotic) effect of tyramine and histamine

The cytotoxic mode of action of tyramine and histamine was further assessed using the LDH assay. LDH is a cytosolic enzyme released into the medium after necrotic cell death. Based on the RTCA results, an LDH assay endpoint of 24 h was chosen. Assays were performed using the concentration range employed to construct the dose-response curves for tyramine and histamine. Figure 5 shows the percentage cytolysis induced by tyramine and histamine. Negligible LDH activity was detected in cell cultures exposed to histamine, except at the highest concentration tested (127.9 mM) in which cytolysis reached 45%. In contrast, a dose-dependent increase in LDH leakage was observed with tyramine, indicating that, unlike histamine, tyramine caused the necrosis of the HT29 cells.

4. Discussion

Dose-dependent toxic effects were observed both in proliferating and confluent cell cultures exposed to either tyramine or histamine. However, tyramine was more cytotoxic than histamine at all time points, as indicated by

its lower IC₅₀, NOAEL and LOAEL values compared to histamine. To our knowledge, this is the first report of IC₅₀, NOAEL and LOAEL values for tyramine and histamine recorded for *in vitro* cultures of intestinal cell lines. Tyramine had an acute, rapid effect; the cell index dropped abruptly after treatment with 14.4 mM tyramine or higher doses, whereas a similar dose of histamine induced more moderate effects. An explanation for this can be found in the different mode of action of tyramine and histamine. LDH release from tyramine-exposed cells increased significantly, particularly at higher concentrations, indicating that this BA has a necrotic effect. In contrast, negligible cytolytic activity was detected in cultures exposed to histamine, suggesting that this BA causes cell death without affecting membrane stability. In addition, cells exposed to histamine showed significantly increased DNA fragmentation, strongly suggesting that the biochemical event behind the cell death induced by it is apoptosis rather than necrosis. Whereas necrosis is an unexpected, unordered and accidental form of cell death, apoptosis is a form of programmed cell death (Kanduc, Mittelman, Serpico, Sinigaglia, Sinha, Natale, et al., 2002). It has been reported that histamine is deeply involved in apoptosis triggering and progression (Toninello, Salvi, Pietrangeli, & Mondovi, 2004). Histamine is a key mediator in the development of allergic reactions, which evoke activation and degranulation of eosinophils and airway epithelial tissue injury; apoptosis is believed to be a major mechanism for the clearance of eosinophilic inflammation (Hasala, Giembycz, Janka-Junttila, Moilanen, & Kankaanranta, 2008; Ling, Ngo, Nguyen, Thurmond, Edwards, Karlsson, et al., 2004).

369 The *in vitro* approach used in this work was shown to be a useful way of
370 assessing the risk of toxicity after the consumption of food containing
371 tyramine and histamine. These compounds are the most toxic of all BAs, and
372 of particular importance with respect to food safety (European Food Safety
373 Authority, 2011). However, in Europe legal limits have only ever been set for
374 histamine, and then only in fish and fish products (European Commission,
375 2005; established by the EFSA), and in the USA, the FDA has only
376 established a guidance histamine concentration of 50 mg/kg for the
377 consumption of scombroid or scombroid-like fish (Food and Drug
378 Administration, 2001). There is no legal limit at all for histamine in other
379 foods, yet it can reach concentrations of 1850 mg/kg in cheese, 400 mg/kg in
380 fermented sausages, and 758 mg/kg in sauces (European Food Safety
381 Authority, 2011). These levels are much higher than the cytotoxicity threshold
382 detected in the present work for histamine (4.0 mM, equivalent to 440.6
383 mg/kg). This result supports previous assumptions that concentrations of
384 histamine above 400 mg/kg are probably dangerous to health (Taylor, 1985).
385 More recently, other authors have suggested histamine concentration of 500
386 mg/kg in food to be hazardous to human health (Askar & Treptow, 1986;
387 Rauscher-Gabernig, Grossgut, Bauer, & Paulsen, 2009; Shukla, Kim, & Kim,
388 2011). It is generally assumed that histamine is the most toxic BA; however,
389 to our knowledge there are no scientific data to substantiate it. The EFSA
390 considers tyramine and histamine as the most toxic BA, but indicates that
391 there is no available information to conduct quantitative risk assessment of
392 BA (European Food Safety Authority, 2011). No legal limit for tyramine in
393 food has ever been set anywhere, yet the present results indicate that this

BA is even more toxic than histamine. The recorded cytotoxicity threshold of 2.2 mM is equivalent to 301.8 mg/kg, a concentration easily exceeded in cheese (in which concentrations of 2519 mg/kg have been detected (Redruello, Ladero, Cuesta, Alvarez-Buylla, Martin, Fernandez, et al., 2013), fermented sausages (up to 1740 mg/kg), fish and fish products (up to 634 mg/kg) and fish sauces (up to 741 mg/kg) (European Food Safety Authority, 2011). Concentrations below this threshold might also cause adverse reactions, although not so severe. However, people with less powerful BA-detoxification systems caused by genetic deficiencies, gastrointestinal disease, mono- or diamine oxidase inhibitor medication, or who have ingested alcohol or other potentiating factors (Maintz & Novak, 2007), might be at greater risk.

In summary, the present results reveal that unexpectedly, tyramine was more cytotoxic than histamine on an *in vitro* model of the human intestinal epithelium. Nevertheless, in both cases the concentrations found to be toxic are commonly reached in BA-rich foods. Thus, it might be advisable to establish measures aimed at reducing their concentrations to below the cytotoxic levels here determined.

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6. Figure legends

Figure 1. Real-time cell analysis of the effects of tyramine and histamine on HT29 cells. Cells were seeded in a 16-well E-plate (20,000 cells/well) and after 20 h of growth were exposed to the indicated concentrations of tyramine (A) or histamine (B) or control medium (0 mM). The point of administration of tyramine or histamine is shown by vertical arrows on the graph. Changes in the normalized cell index were dynamically monitored every 15 min for 24 h. Data are the means of three replicates; vertical bars represent standard deviations.

Figure 2. Dose-response curves for tyramine and histamine in HT29 intestinal cells. Cell cultures were treated with a range of tyramine (A) or histamine (B) concentrations for 24 h. Data represent the means $\pm$ standard deviations of three experiments. The non-linear regression trend lines fitted a sigmoid dose-response curve (variable slope). An asterisk indicates the first significant difference with respect to the minimal dose of BA assayed (0.7 and 0.2 mM for tyramine and histamine respectively) and represents the LOAEL concentration (* $p < 0.05$). Numeric values for NOAEL, LOAEL, Hill slope and R^2 are also indicated.

Figure 3. Toxicity of tyramine and histamine on intestinal cells. HT29 cells were grown in flat-bottomed microplates for 20 h, and then treated with increasing concentrations of (A) tyramine or (B) histamine for 24 h. They were then fixed, stained with DAPI, and viewed with an epifluorescence

microscope. Apoptotic cells are marked with white arrows.

Figure 4. Apoptotic DNA-fragmentation induced by tyramine and histamine in HT29 intestinal cells. BrdU-labelled DNA fragments released from the nucleus to the cytoplasm due to apoptosis were quantified in HT29 cells exposed to 5.8 mM of tyramine or 16.3 mM histamine for 24 h. The percentage of DNA fragmentation was calculated as follows: $100 \times [(DNA \text{ fragmentation of treated samples} - DNA \text{ fragmentation of negative control}) / (DNA \text{ fragmentation of positive control} - DNA \text{ fragmentation of negative control})]$. The data represent the means of three replicates; vertical bars represent standard deviations.

Figure 5. Determination of the cytolytic effect of tyramine (A) and histamine (B) on intestinal cells. HT29 cells were seeded in 96-well plates and after 20 h of growth in different concentrations of the corresponding BA were added to each well incubated for 24 h. Necrosis was then measured via lactate dehydrogenase activity (LDH). The data represent the percentage of cells lysed by treatment with different concentrations of tyramine or histamine. The percentage of cells lysed was calculated as follows: $100 \times [(492 \text{ nm absorbance of treated samples} - \text{absorbance of negative control}) / (\text{absorbance of positive control} - \text{absorbance of negative control})]$. Data represent the means of three replicates; vertical bars represent standard deviations.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1. IC₅₀ values (mean ± standard deviation) for tyramine and histamine after exposure of HT29 intestinal cells for different times.

Time (h)	Tyramine (IC ₅₀)	Histamine (IC ₅₀)
8	6.9 ± 0.30	261.9 ± 147.50
12	5.0 ± 0.10	55.4 ± 6.30
18	3.4 ± 0.04	31.0 ± 1.90
24	3.2 ± 0.04	26.0 ± 1.20

Values are the mean ± standard deviation in mM