Supporting Information

Imaging Histamine in Live Basophils and Macrophages with a Fluorescent Mesoionic Acid Fluoride

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1. General methods.

Unless stated otherwise, all reactions were carried out under argon in dried glassware. Commercially available reactants were purchased by Sigma-Aldrich and used without further purification. Thapsigargin was supplied by TOCRIS. Thin-layer chromatography was conducted on Merck silica gel 60 F254 sheets and visualized by UV. Silica gel (particle size 35-70 μm) was used for flash column chromatography. ^{1}H and ^{13}C-NMR spectra were recorded with a Varian Mercury 400 spectrometer (400 and 100 MHz respectively) in $CDCl_3$ or DMSO-d_6 solution with TMS as an internal reference. Data for ^{1}H-NMR spectra are reported as follows: chemical shift (δ/ppm), multiplicity, coupling constant (Hz) and integration. Data for ^{13}C-NMR spectra are reported in terms of chemical shift relative to the solvent peak of $CDCl_3$ set at δ = 77.0 ppm. Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Krebs-Ringer HEPES (KR-HEPES) buffer was prepared with HEPES 10 mM, NaCl 120 mM, KCl 4.7 mM, $CaCl_2$ 2.2 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, glucose 1.0 mM, and adjusted to pH 7.3. Spectroscopic data were measured on a SpectraMax M2 plate reader (Molecular Devices), and the data analysis was performed using GraphPad Prism 5.0. Cell viability assays were performed using a Cell Titer 96 Aqueous non-radioactive cell proliferation assay kit (Promega). Fluorescence microscopy experiments were performed in a Nikon A1R confocal microscope, and images were processed using the software NIS-Elements 3.2.

Synthesis and characterization of mesoionic isoquinoline derivatives. Mesoionic isoquinoline adducts (**4a-4h** and **5a-5d**, Scheme S1) were prepared according to the methods described in the literature. Detailed experimental protocols and spectroscopic data can be found at the original reference, together with copies of their NMR spectra.[S1]

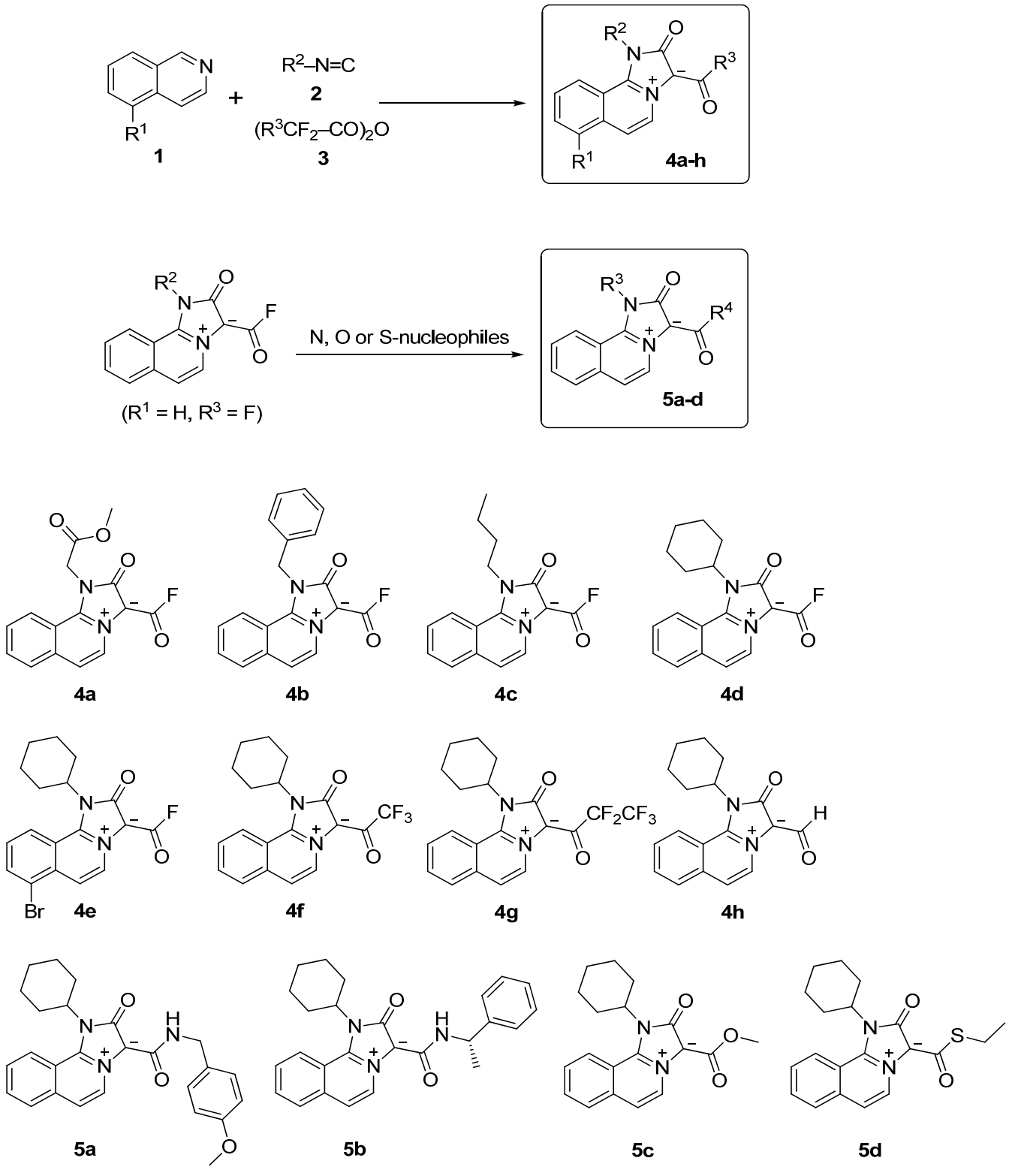
Evaluation of fluorescent properties. Fluorescence spectra were measured using a SpectraMax M2 plate reader in 96 or 384-well plates. Mesoionic isoquinolines were dissolved to a final concentration of 10 μM

in PBS or HEPES buffer containing 1% DMSO, and incubated with the corresponding biomolecules. The excitation wavelength was set at 370 nm unless otherwise stated. Fluorescence increase ratios were determined by referring the maximum fluorescence intensity in the presence of the screened biomolecules to the maximum fluorescence intensity in their absence.

Cell culture and imaging. RAW 264.7 and RBL-2H3 cells were purchased from American Type Culture collection (ATCC) and maintained in DMEM supplemented with 10% or 15% fetal bovine serum (FBS) respectively and antibiotics (100 U mL^{-1} penicillin/100 μg mL^{-1} streptomycin). For live cell imaging, 5×10^5 cells were seeded in 35-mm glass bottom dishes 24 h before the experiment. Cells were washed with Krebs-Ringer HEPES (KR-HEPES) buffer and treated with **Histamine Blue** (20 μM) for 15 min at 37 ºC. After incubation, cells were washed twice with KR-HEPES buffer and imaged under a Nikon A1R confocal microscope. All images were processed using the software NIS-Elements 3.2.

Chemical treatment of RAW 264.7 macrophages. a) Histamine uptake: 5×10^5 RAW 264.7 cells were incubated in 35-mm glass bottom dishes 24 h before the experiment. Cells were incubated with media and histamine at the concentration and time indicated. After washing, cells were treated with **Histamine Blue** (20 μM) for 15 min at 37 ºC, washed twice with KR-HEPES buffer and imaged under the microscope. B) Stimulation with thapsigargin: 3×10^5 RAW 264.7 cells were incubated in 35-mm glass bottom dishes 24 h before the experiment. Cells were incubated with media and thapsigargin (300 nM) for 24 h. After washing, cells were treated with **Histamine Blue** (20 μM) for 15 min at 37 ºC, washed twice with KR-HEPES buffer and imaged under the microscope. The in vitro quantification of histamine levels was performed according to reported procedures.[S2, S3]

2. Chemical structures and characterization of mesoionic isoquinolines.



Scheme S1. Synthetic scheme and chemical structures of mesoionic isoquinolines.

Table S1. Fluorescent properties of isoquinolines **4a-h** and **5a-d**.*

Compound code	λabs	λem	ϕ
4a	346	417	0.196
4b	341	418	0.385
4c	345	418	0.348
4d	351	420	0.201
4e	361	443	0.032
4f	370	402	0.001
4g	370	419	0.001
4h	378	420	0.006
5a	363	454	0.464
5b	362	451	0.494
5c	346	441	0.354
5d	371	430	0.390

* Absorbance and emission maxima values were measured in DMSO. Quantum yields (ϕ) in DMSO (excitation wavelength: 370 nm) were determined as reported elsewhere[S4] using coumarin as a reference (ϕ: 0.68).[S5]

3. Fluorescent response of mesoionic isoquinolines against histamine.

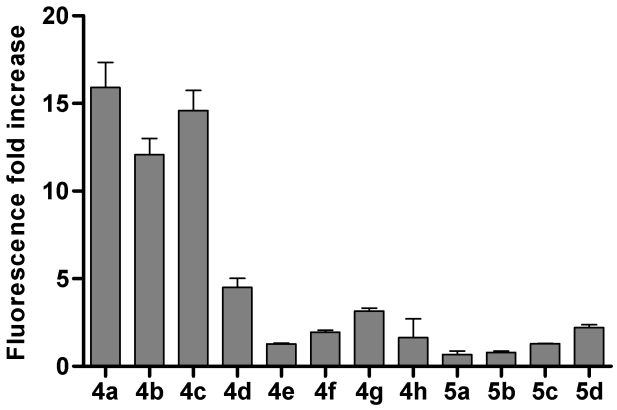


Figure S1. Fluorescence increase of mesoionic isoquinolines (**4a-4h** and **5a-5d**, 10 μM) upon incubation with histamine (5 mM) for 45 min in PBS buffer (pH 7.3). Excitation wavelength: 370 nm. Values are represented as means (*n*=3) and errors bars as standard deviations.

4. HPLC-MS analysis of the reaction between 4a and histamine.

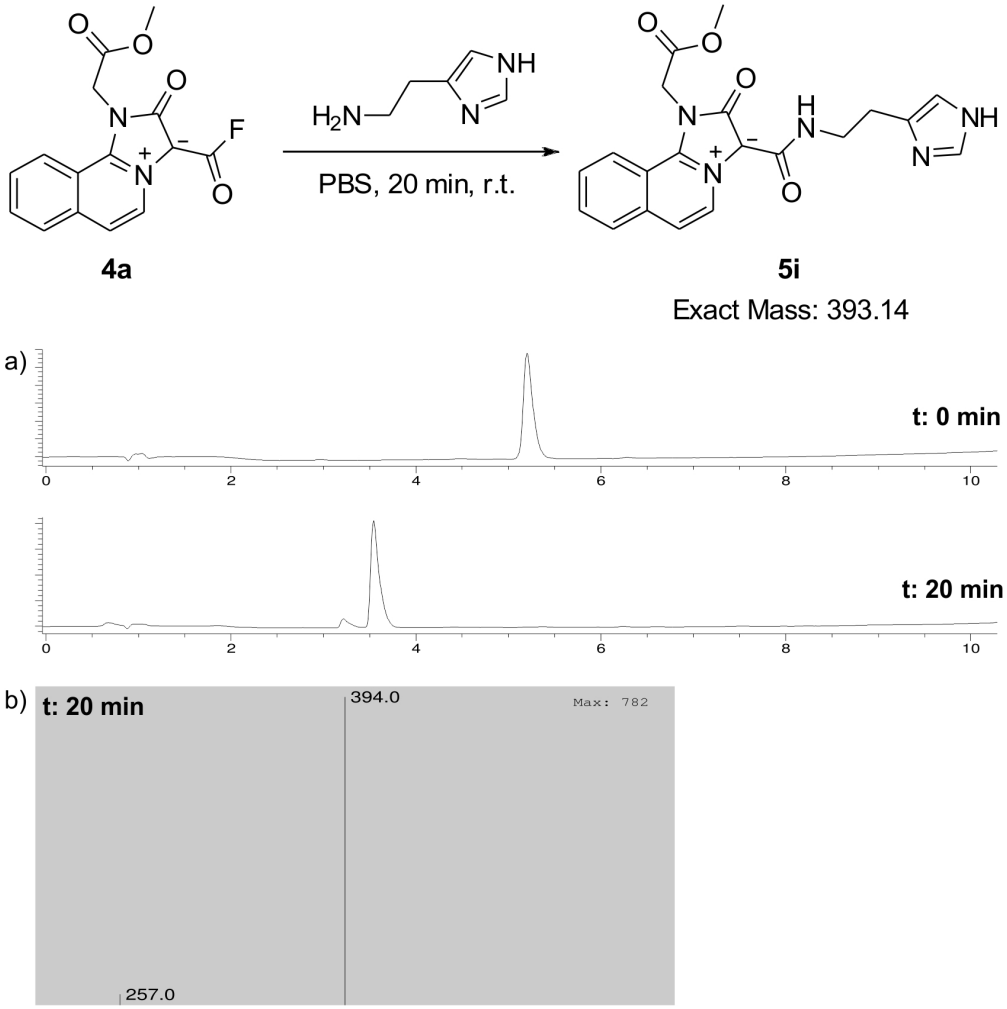
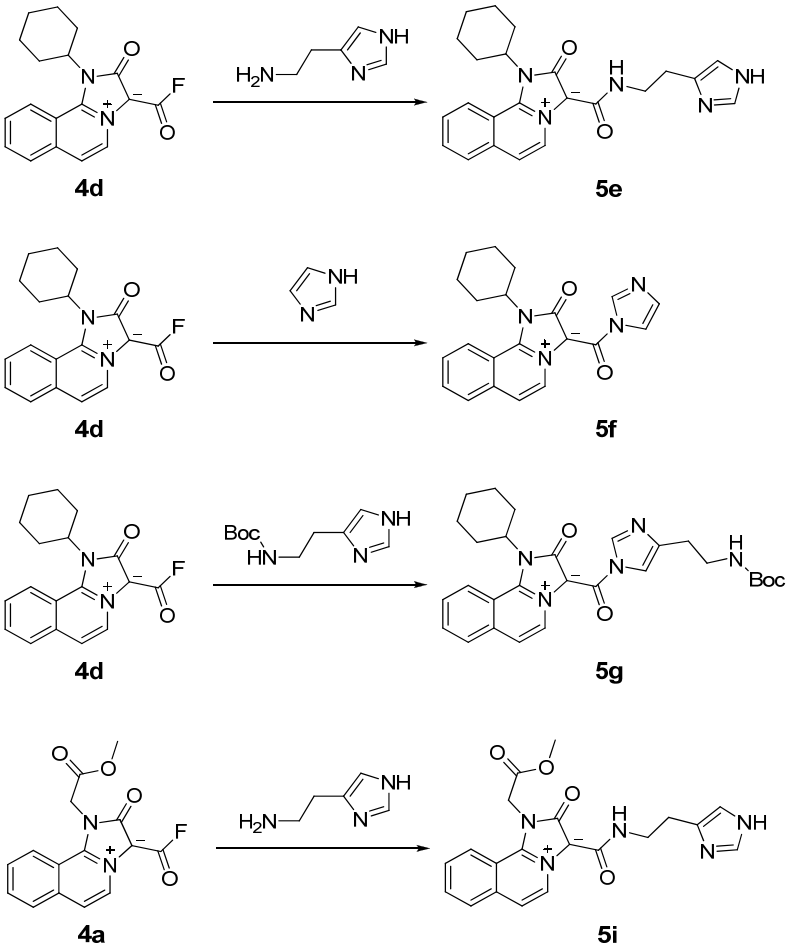


Figure S2. HPLC-MS analysis of the reaction between **4a** and histamine in PBS buffer. a) Chromatograms at 350 nm, b) MS spectra of the main product obtained after 20 min reaction. HPLC conditions: A: H_2O-HCOOH: 99.9:0.1, B: ACN-HCOOH: 99.9:0.1; gradient 5% B to 95% B (10 min), C_{18}(2) Luna column (4.6 × 50 mm^2, 5 μm particle size), flow rate: 1 mL/min.

5. Spectral characterization of histamine adducts.

We synthesized and characterized all possible adducts of the mesoionic acid fluorides with histamine and imidazole (**Scheme S2**). This study was initially carried out with the acid fluoride **4d** since it was available in multigram quantities, and later confirmed with the acid fluoride **4a**.



Scheme S2. Synthesized adducts resulting from the reaction between mesoionic acid fluorides and histamine, imidazole and *N*-Boc-histamine.

5e-5g were prepared following similar reported procedures[S1] by using histamine, imidazole and *N*-Boc-histamine as nucleophiles respectively. *N*-Boc-histamine was prepared using the methodology described by Qu and co-workers.[S6] Analogously, the reaction of **4a** with histamine rendered the adduct **5i**.

Absorbance and emission spectra of **5e-5i** were compared to those obtained after incubating the acid fluorides **4d** and **4a** with histamine in PBS (Figure S5). The spectra of **5e** (but not those of **5f** or **5g**) resembled those obtained after the reaction of **4d** with histamine, proving that that the linkage between **4d**

and histamine was through the primary amine group and not through the nitrogen of the imidazole ring. The same observation was corroborated with **4a** and **5i** (Figure S3).

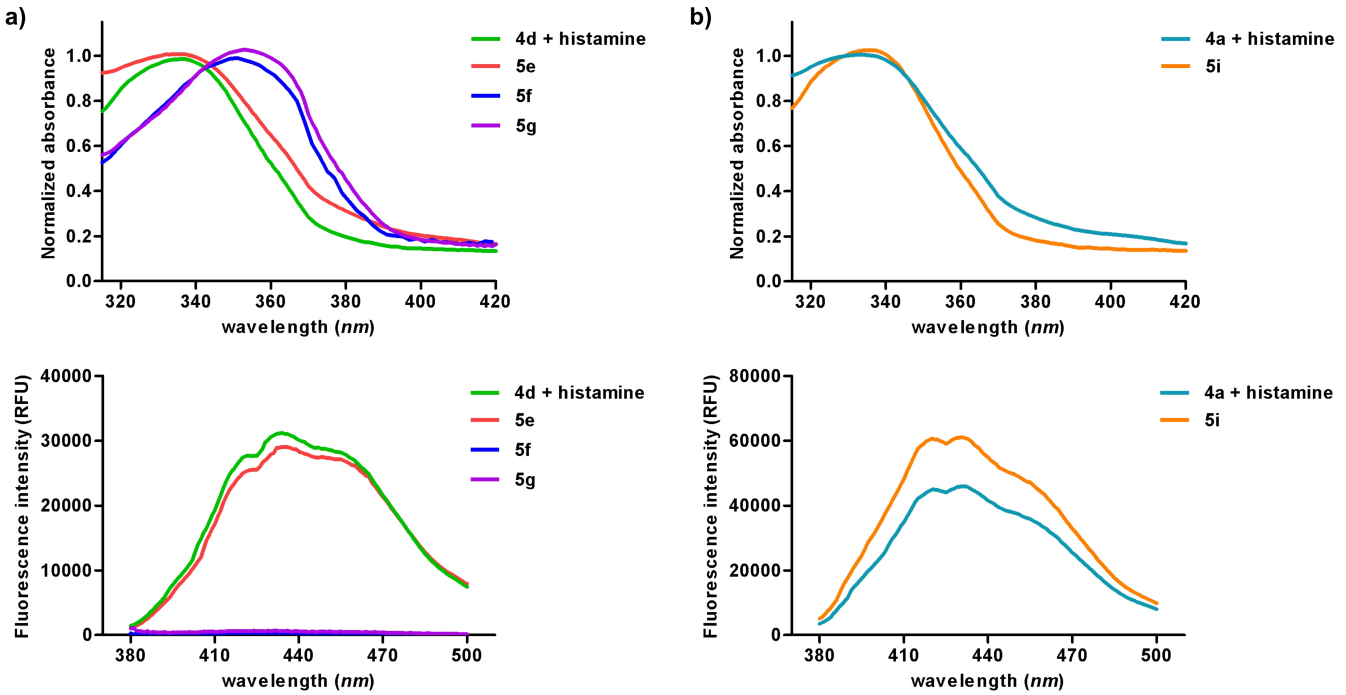
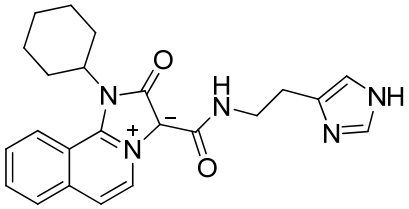


Figure S3. Spectral characterization of histamine adducts. a) Absorbance (*upper*) and fluorescence (*lower*) spectra of adducts **5e**, **5f** and **5g** (100 µM in PBS, 2% DMSO) and the acid fluoride **4d** (100 µM) after reaction with histamine in PBS, b) absorbance (*upper*) and fluorescence (*lower*) spectra of **5i** (100 µM in PBS, 2% DMSO) and **4a** (100 µM) after reaction with histamine in PBS. Excitation wavelength: 350 nm.

6. NMR and MS characterization of histamine adducts.

3-(2-(1H-Imidazol-4-yl)ethylcarbamoyl)-1-cyclohexyl-2-oxo-2,3-dihydro-1H-imidazo[2,1-a]isoquinolin-4-ium-3-ide (5e)



5e

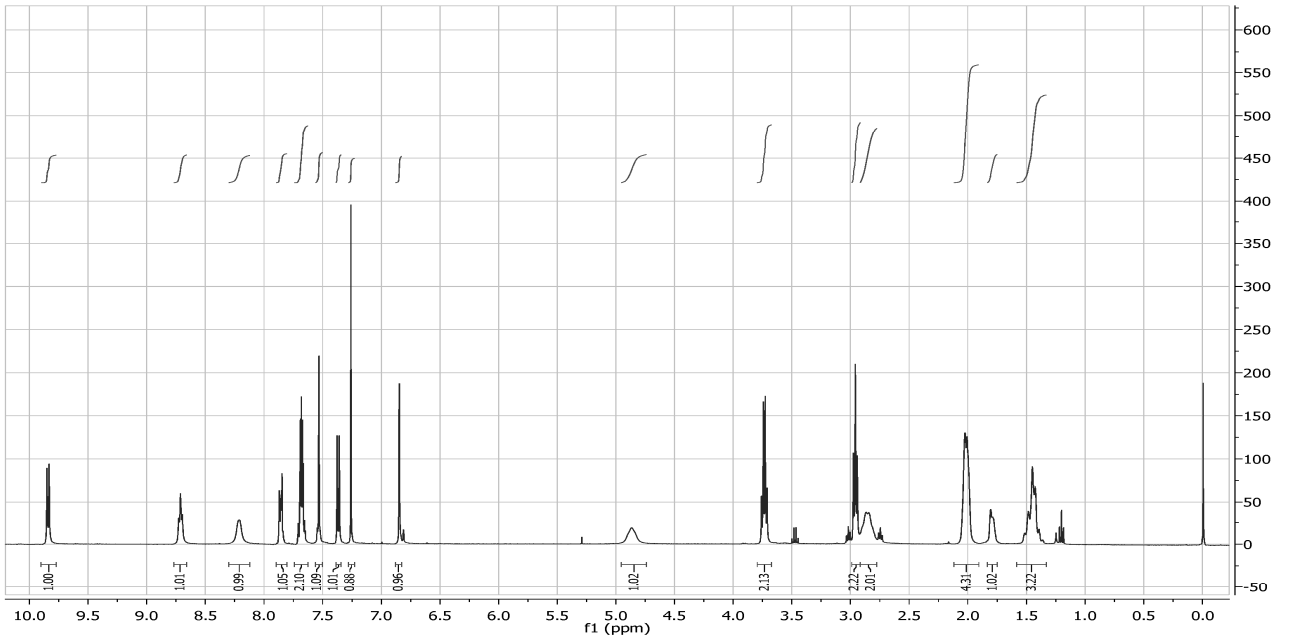
Obtained as a yellow solid (95%), purified by precipitation (CH_2Cl_2/Et_2O).

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.84 (d, *J* = 7.2 Hz, 1H), 8.71 (t, *J* = 5.9 Hz, 1H), 8.21 (s, 1H), 7.88 – 7.83 (m, *J* = 9.4 Hz, 1H), 7.72 – 7.63 (m, *J* = 6.5, 2.7 Hz, 2H), 7.53 (s, 1H), 7.37 (d, *J* = 7.2 Hz, 1H), 6.85 (s, 1H), 4.86 (s, 1H), 3.73 (q, *J* = 6.6 Hz, 2H), 2.96 (t, *J* = 6.6 Hz, 2H), 2.91 – 2.80 (m, *J* = 9.0 Hz, 2H), 2.02 (d, *J* = 6.7 Hz, 4H), 1.82 – 1.76 (m, *J* = 6.7 Hz, 1H), 1.53 – 1.37 (m, *J* = 12.8 Hz, 3H), one mobile proton not detected.

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 162.82, 158.84, 134.83, 131.88, 129.49, 129.22, 128.74, 128.63, 124.25, 122.58, 116.69, 115.26, 96.50, 86.28, 58.94, 37.98, 30.00, 27.72, 26.40, 25.14.

UV (DMSO) λmax (log_{10} ε): 369 nm (4.17).

MS (EI) m/z (%): 403 (M+, 13), 322 (39), 293 (65), 227 (19), 211 (100), 184 (18), 155 (31), 128 (14), 81 (9), 55 (10); HRMS: calcd for $C_{23}H_{26}N_5O_2^+$ 404.2027, found 404.2081.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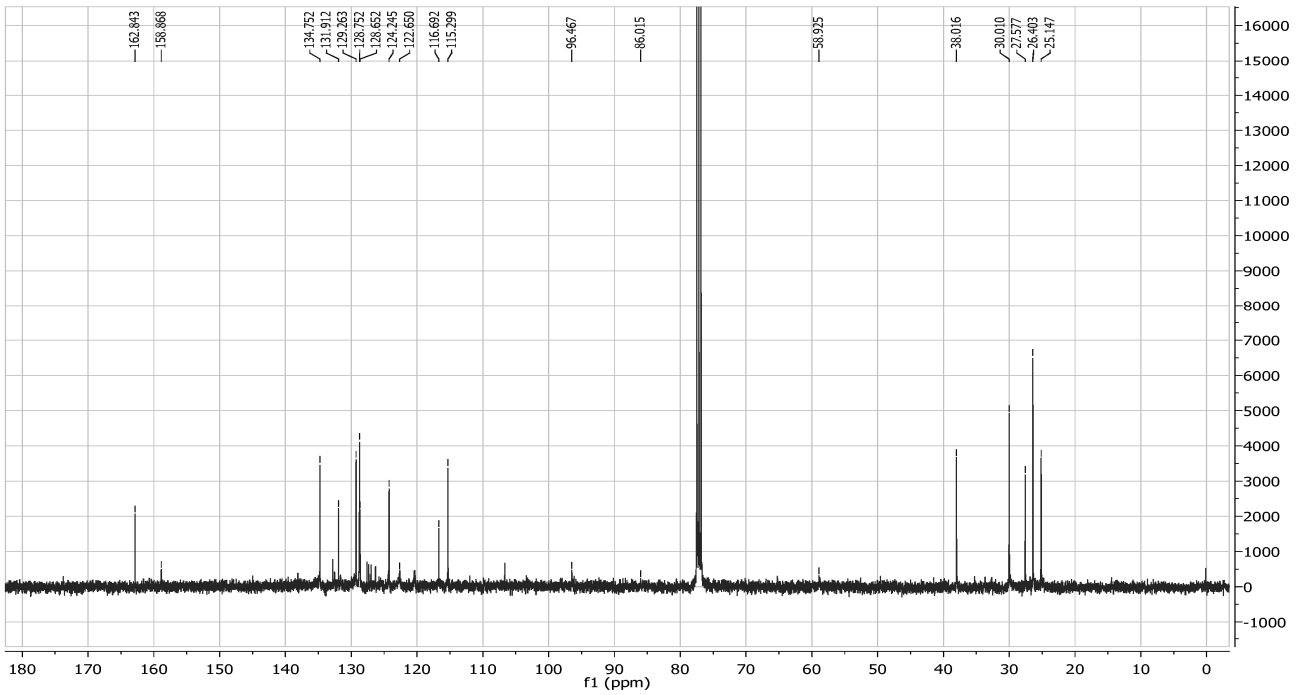
1.02
3.22
f1 (ppm)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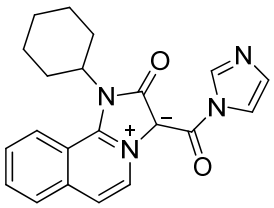
58.925
38.016
30.010
27.577
26.403
25.147
f1 (ppm)

1-Cyclohexyl-3-(1H-imidazole-1-carbonyl)-2-oxo-2,3-dihydro-1H-imidazo[2,1-a]isoquinolin-4-ium-3-ide (5f)



5f

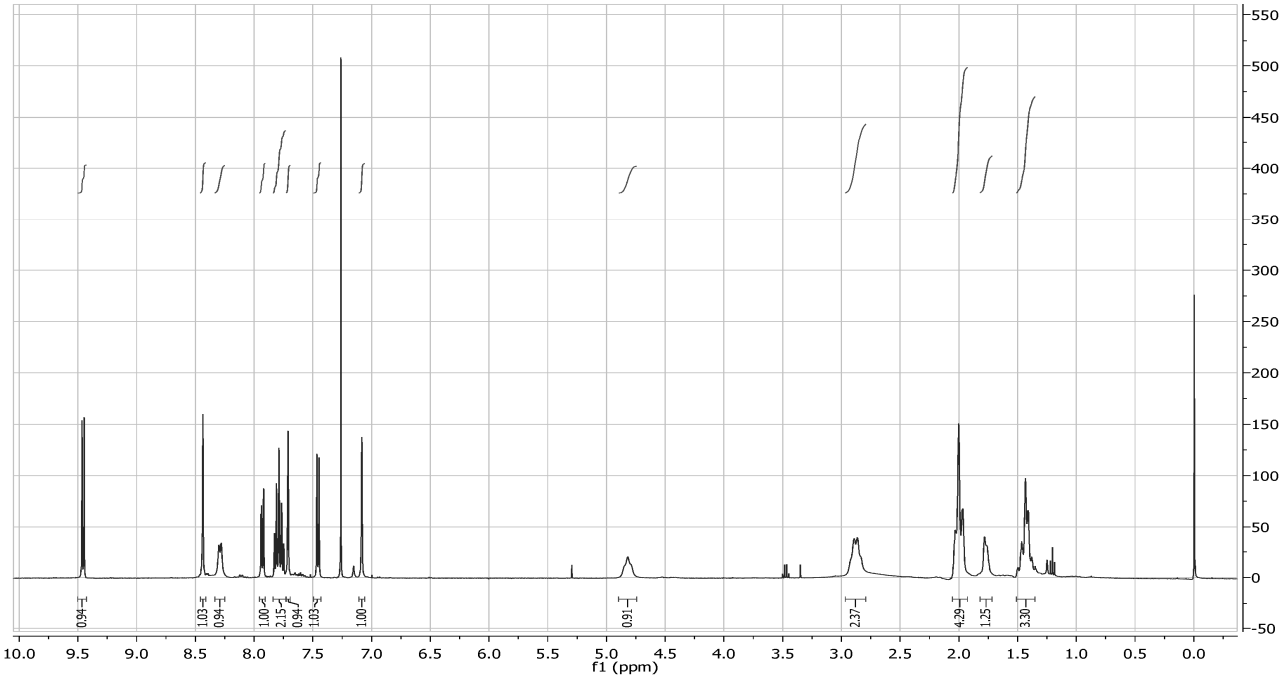
Obtained as a yellow solid (90%), purified by precipitation (CH_2Cl_2/Et_2O).

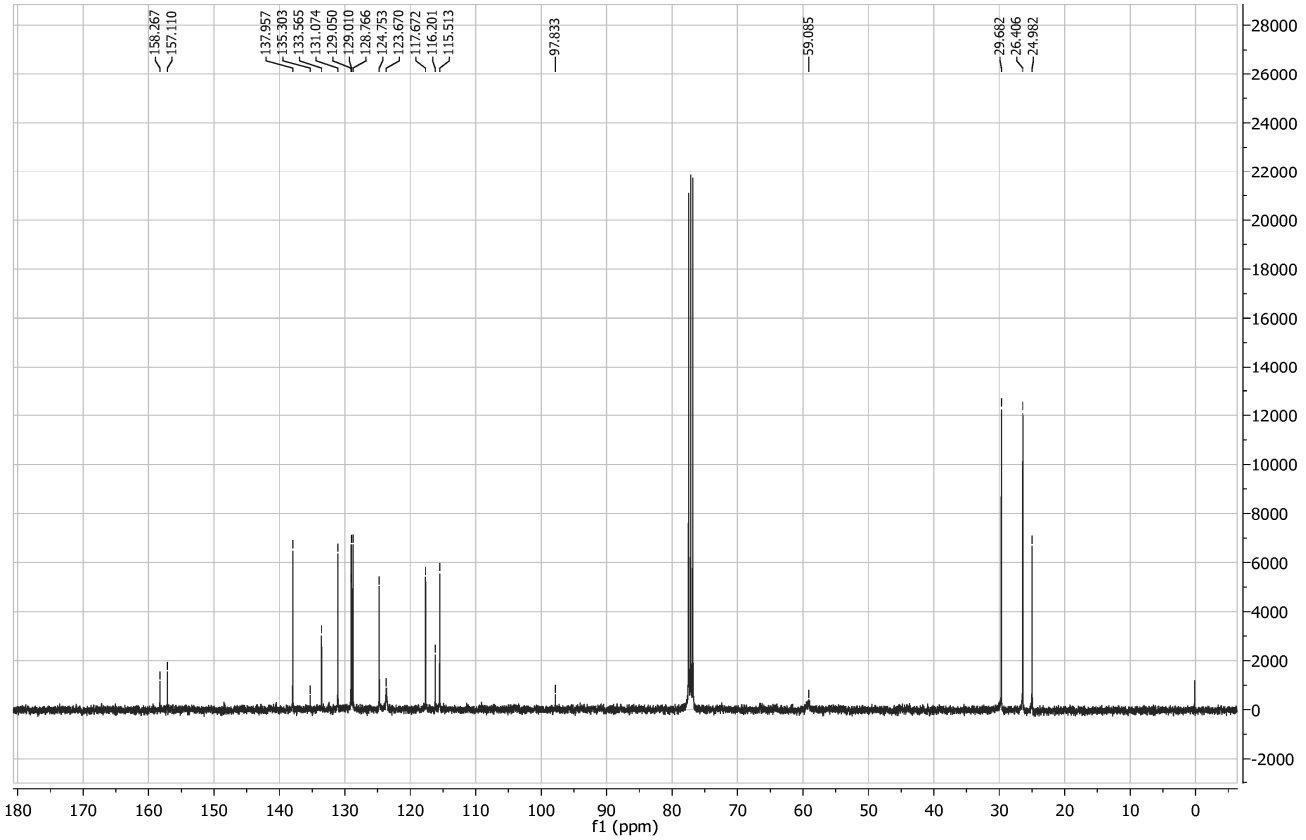
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.46 (d, *J* = 7.3 Hz, 1H), 8.44 (s, 1H), 8.29 (d, *J* = 7.9 Hz, 1H), 7.95 – 7.91 (m, 1H), 7.84 – 7.74 (m, 2H), 7.71 (s, 1H), 7.46 (d, *J* = 7.3 Hz, 1H), 7.08 (s, 1H), 4.82 (m broad, 1H), 2.88 (m, *J* = 10.7 Hz, 2H), 2.05 – 1.95 (m, 4H), 1.80 – 1.72 (m, 1H), 1.51 – 1.36 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ= 158.35, 157.19, 138.04, 135.38, 133.65, 131.15, 129.13, 129.09, 128.85, 124.83, 123.75, 117.75, 116.28, 115.59, 97.91, 59.17, 29.76, 26.49, 25.06.

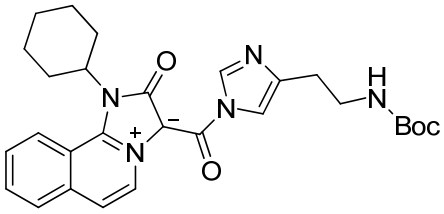
UV (CH_2Cl_2) λmax ($\log_{10}$ ε): 380 nm (4.31).

MS (EI) m/z (%): 360 (M+, 16), 293 (34), 211 (100), 155 (24), 154 (10), 128 (12); HRMS: calcd for $C_{21}H_{21}N_4O_2^+$ 361.1659, found 361.1655.





3-(4-(2-(tert-Butoxycarbonylamino)ethyl)-1H-imidazole-1-carbonyl)-1-cyclohexyl-2-oxo-2,3-dihydro-1H-imidazo[2,1-a]isoquinolin-4-ium-3-ide (5g)



5g

Obtained as a white solid (80%), purified by column chromatography (SiO2, Hexanes /Ethyl acetate).

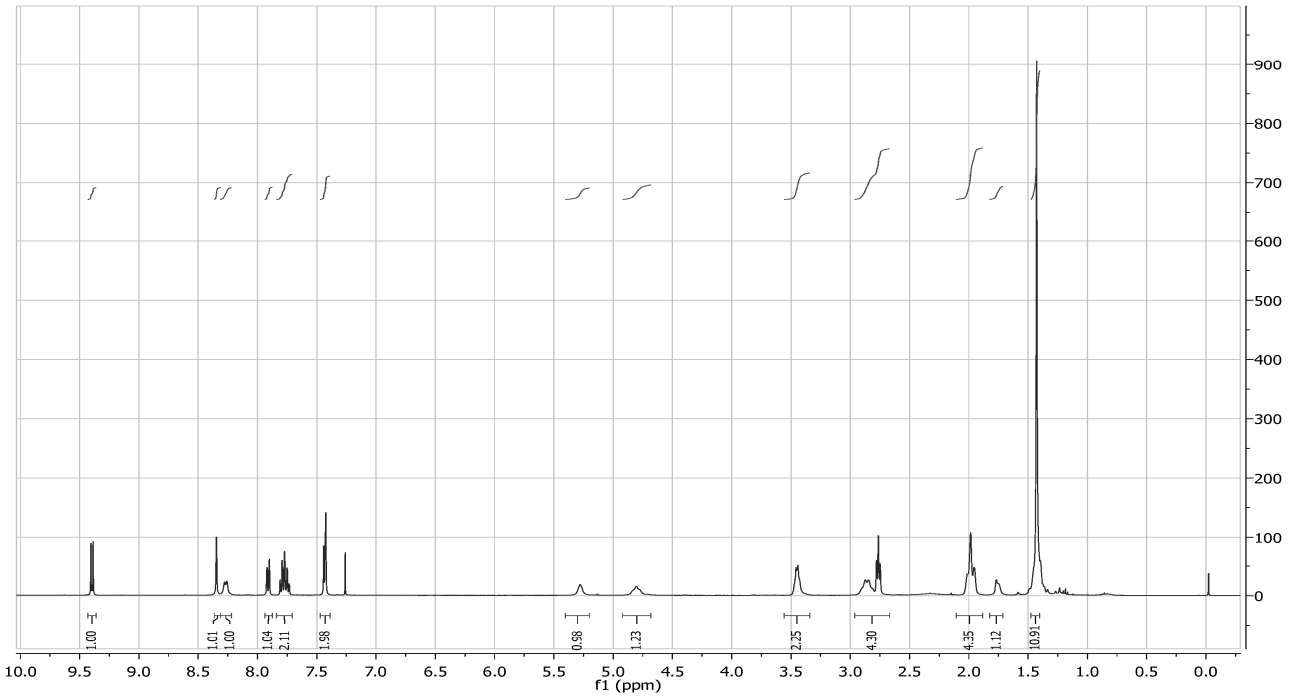
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.43 (d, *J* = 7.3 Hz, 1H), 8.37 (d, *J* = 1.0 Hz, 1H), 8.29 (d, *J* = 7.5 Hz, 1H), 7.96 – 7.90 (m, 1H), 7.79 (m, *J* = 13.7, 7.0, 3.4 Hz, 2H), 7.48 – 7.42 (m, *J* = 7.2 Hz, 2H), 5.28 (s broad, 1H), 4.82 (m broad, 1H), 3.47 (m, *J* = 5.8 Hz, 2H), 2.88 (m, *J* = 10.6 Hz, 2H), 2.79 (t, *J* = 6.3 Hz, 2H), 2.08 – 1.92 (m, 5H), 1.82 – 1.64 (m, 3H), 1.45 (m, 9H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.22, 156.82, 156.15, 139.98, 138.00, 135.14, 133.47, 131.03, 129.03, 128.73, 124.64, 123.60, 116.15, 115.49, 113.95, 97.73, 78.94, 59.25, 40.09, 29.65, 28.57, 28.42, 26.35, 24.93.

UV (CH_2Cl_2) λmax (log ε): 369 nm (4.17).

MS (EI) m/z (%): 503 (M+, 3), 294 (11), 295 (55) 212 (14) 211 (100), 155 (32), 128 (12), 67(11).

HRMS: calcd for $C_{28}H_{34}N_5O_4^+$ 504.2605, found 504.2603.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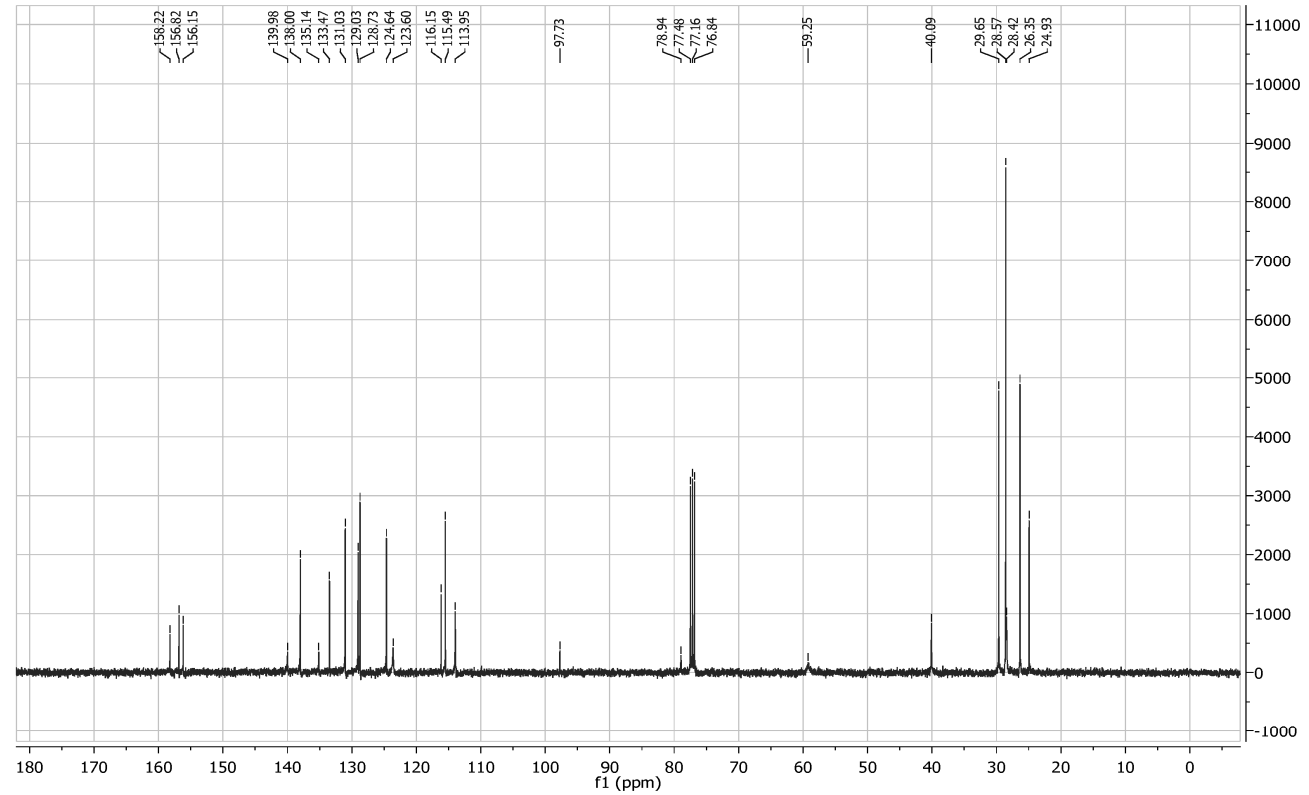
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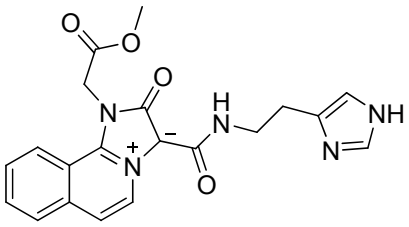
78.94
77.48
77.16
76.84
59.25
40.09
29.65
28.57
28.42
26.35
24.93
f1 (ppm)

3-(2-(1H-imidazol-4-yl)ethylcarbamoyl)-1-(2-methoxy-2-oxoethyl)-2-oxo-2,3-dihydro-1H-imidazo[2,1-a]isoquinolin-4-ium-3-ide (5i)



5i

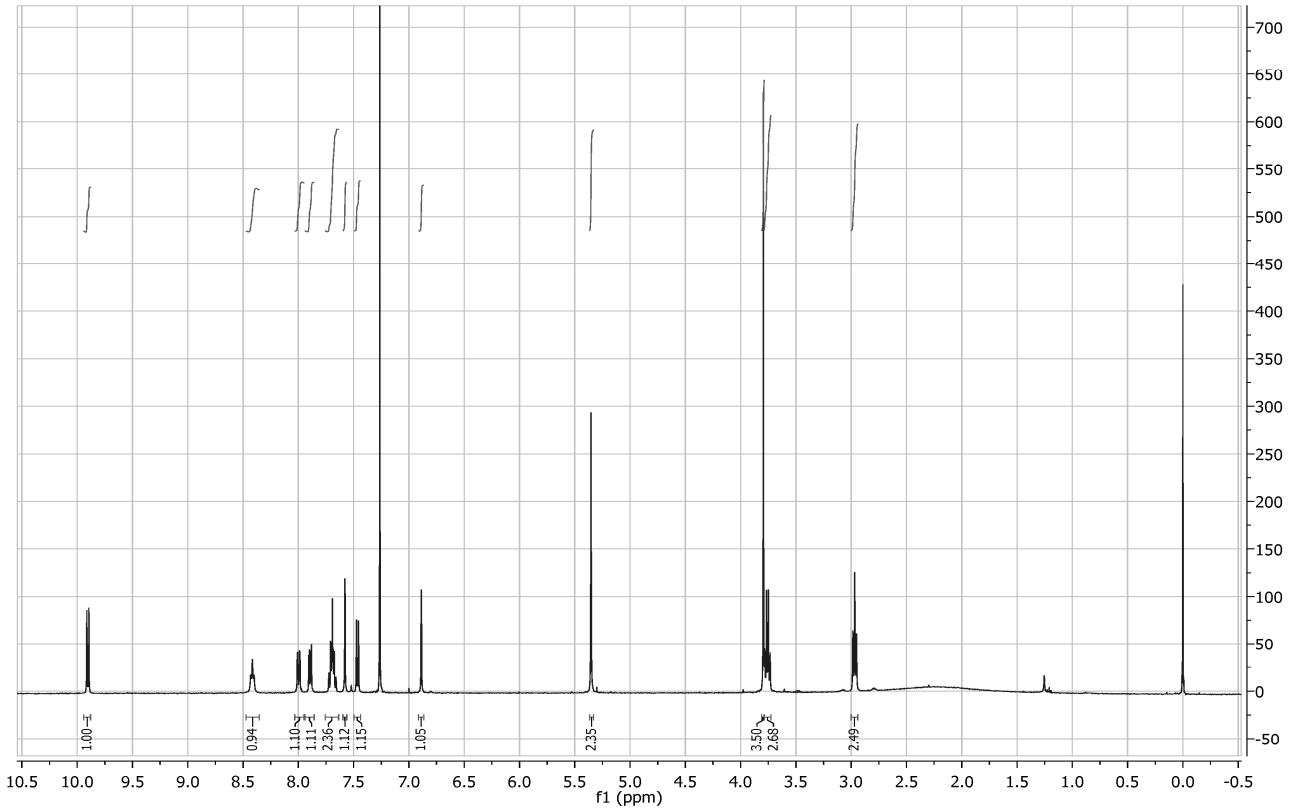
Obtained as a brown solid (90%), purified by precipitation (CH_2Cl_2/Et_2O).

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.90 (d, *J* = 7.3 Hz, 1H), 8.42 (t, *J* = 6.0 Hz, 1H), 8.06 – 7.96 (m, 1H), 7.94 – 7.85 (m, *J* = 6.8, 2.5 Hz, 1H), 7.78 – 7.63 (m, 2H), 7.58 (s, 1H), 7.46 (d, *J* = 7.3 Hz, 1H), 6.89 (s, 1H), 5.35 (s, 2H), 3.79 (s, 3H), 3.78 – 3.73 (m, *J* = 6.5 Hz, 2H), 2.97 (t, *J* = 6.5 Hz, 2H), one broad mobile proton.

^{13}C-NMR (100 MHz, DMSO) δ = 168.30, 161.09, 155.97, 134.78, 134.71, 130.89, 129.77, 129.57, 129.08, 128.26, 123.34, 122.17, 115.97, 115.74, 93.90, 52.90, 42.86, 37.71. One quaternary carbon not detected.

MS (EI) m/z (%): 393 (M+, 4), 376 (12), 312 (13), 299 (16), 283 (60), 269 (12), 242 (40), 197 (56), 169 (39), 128 (34), 115 (19), 95 (91), 81 (100), 54 (17).

HRMS: calcd for $C_{20}H_{20}N_5O_4^+$ 394.1510, found 394.1502.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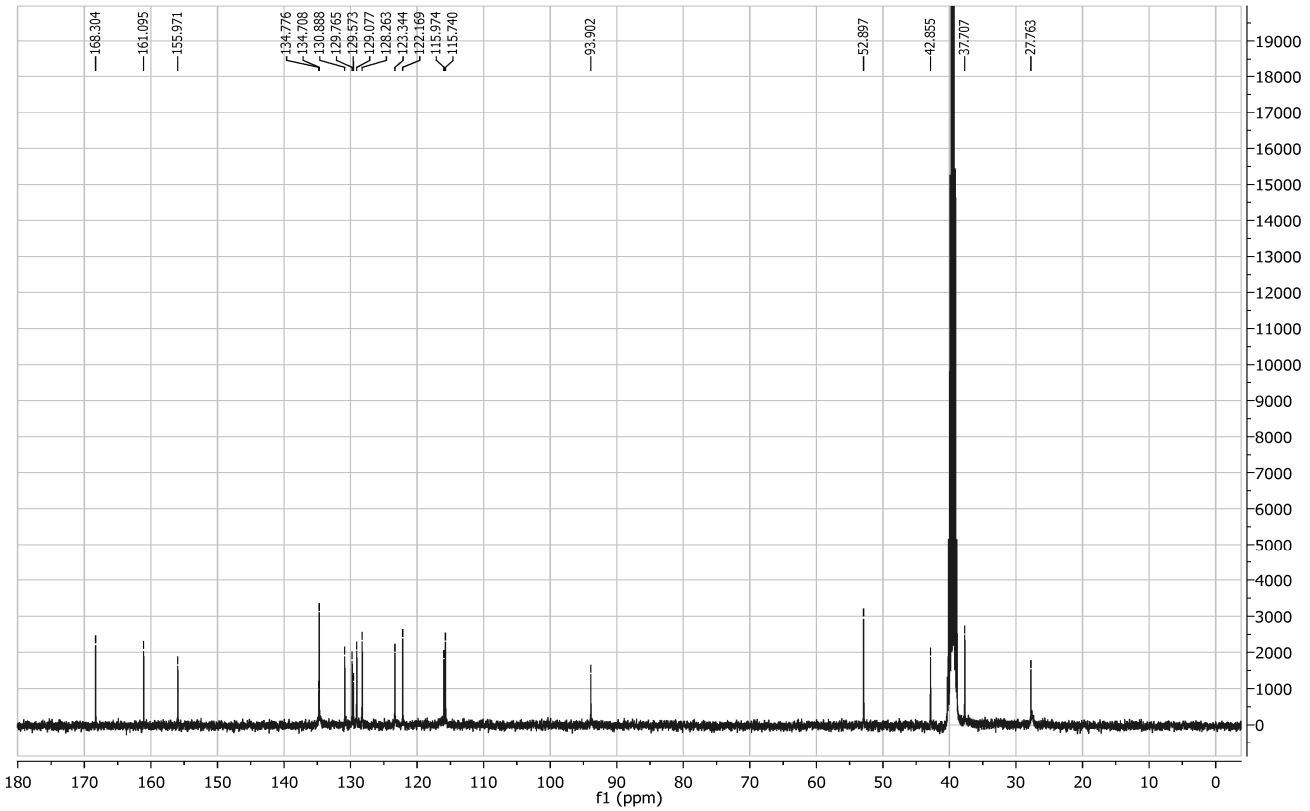
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27.763
f1 (ppm)

7. Fluorescent response of 4a against different biomolecules.

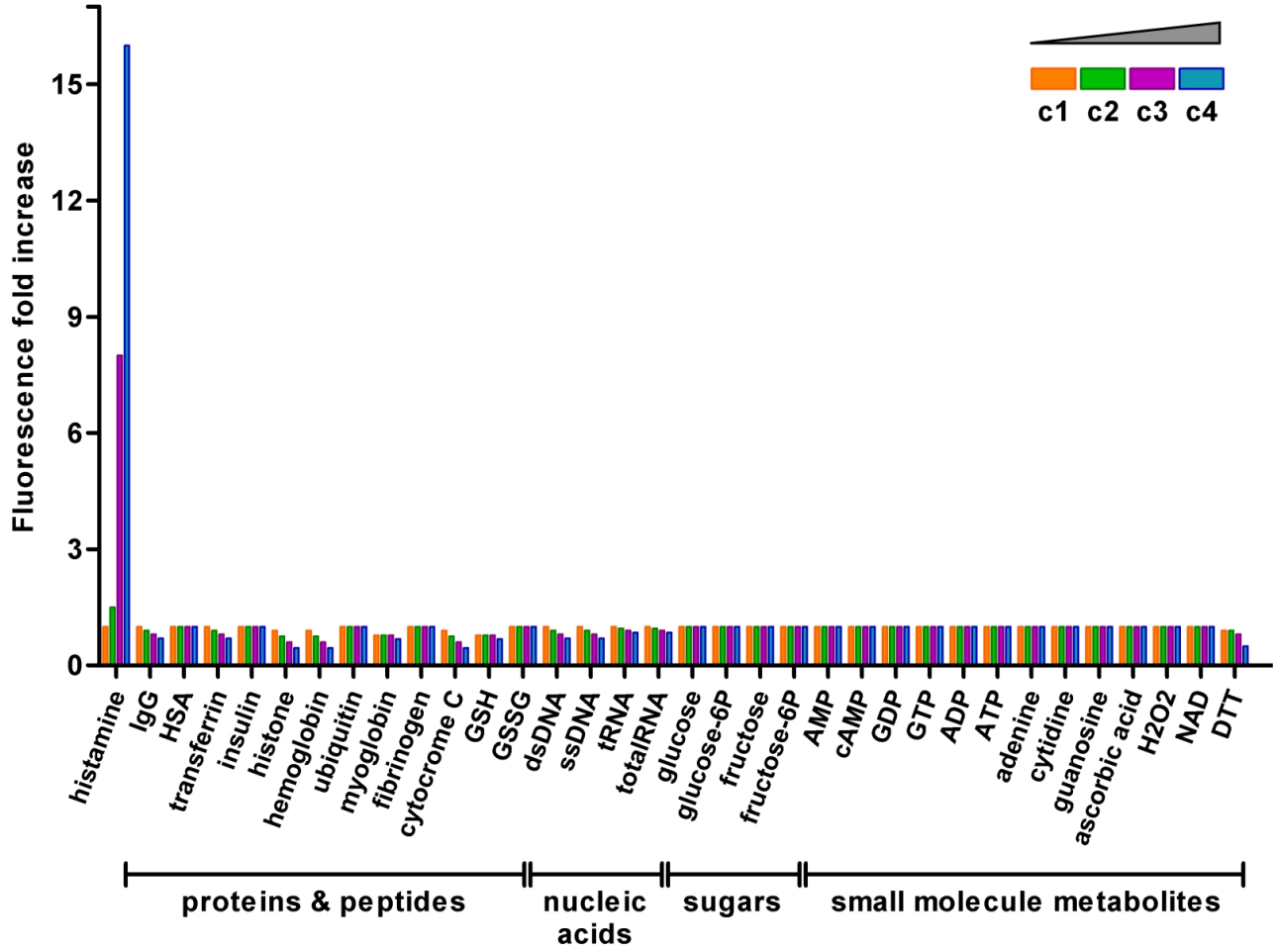


Figure S4. Fluorescent response of **4a** (10 μM) with several biomolecules. Excitation wavelength: 370 nm. Concentrations (c1-c4): proteins, peptides and nucleic acids: 0.125, 0.25, 0.5 and 1 mg/mL; sugars: 0.08, 0.4, 2 and 10 mM; AMP, cAMP, GDP, GTP, ADP, ATP, adenine, cytidine and guanosine: 12.5, 25, 50 and 100 μM; histamine, ascorbic acid, H_2O_2, NAD and DTT: 0.04, 0.2, 1 and 5 mM.

8. Comparative reactivity of 4a (Histamine Blue) with different amines.

We monitored the reactions of **Histamine Blue** with 2-(pyridin-2-yl)ethylamine and 2-phenylethylamine by HPLC. Both reactions proceeded at a similar rate (Figure S5), much slower than the reaction with histamine ($t_{1/2}$: aprox. 8 min), suggesting that the role of the sp^2 nitrogen of the heterocycle was not the main factor in controlling the rate determining step.

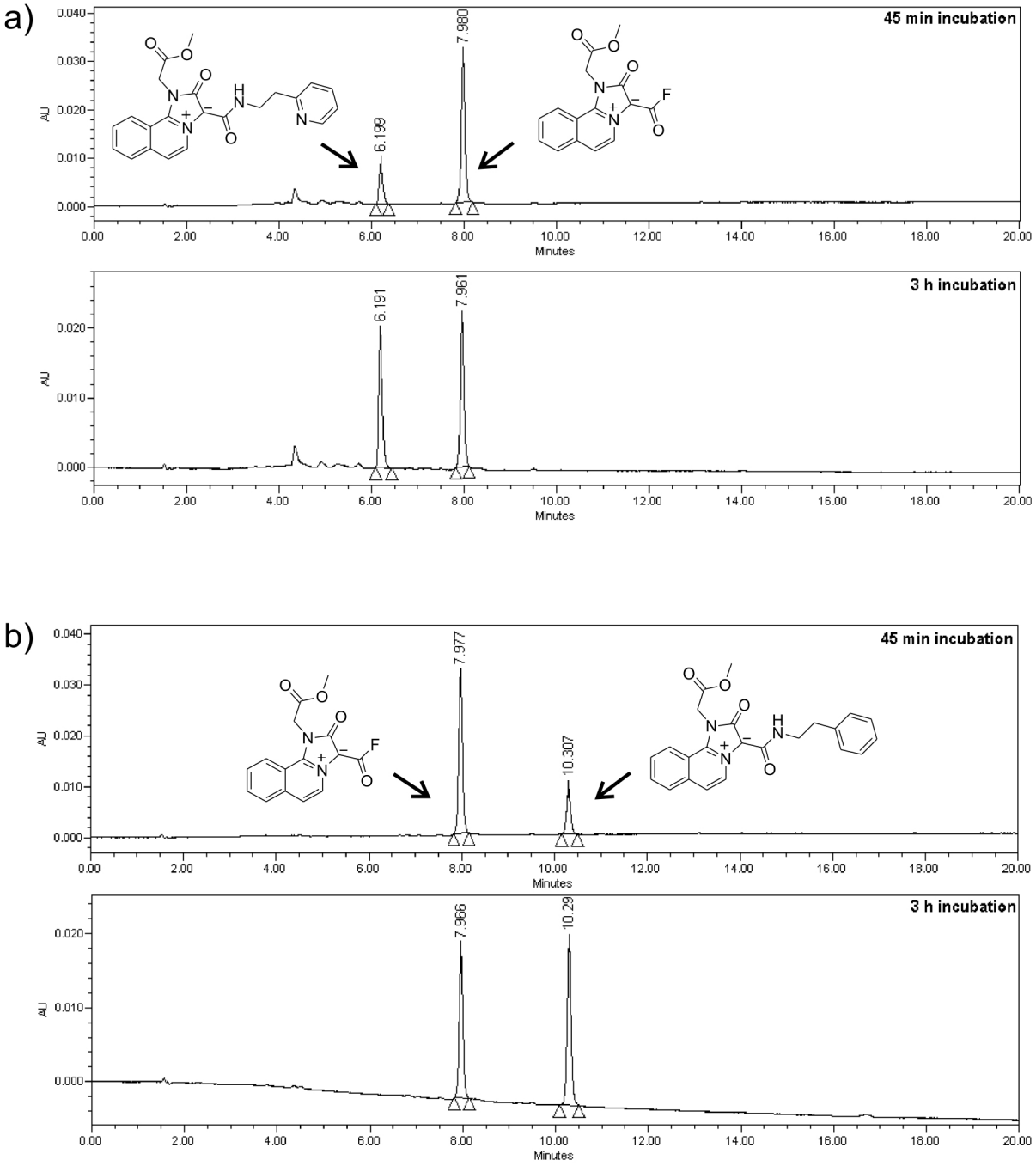
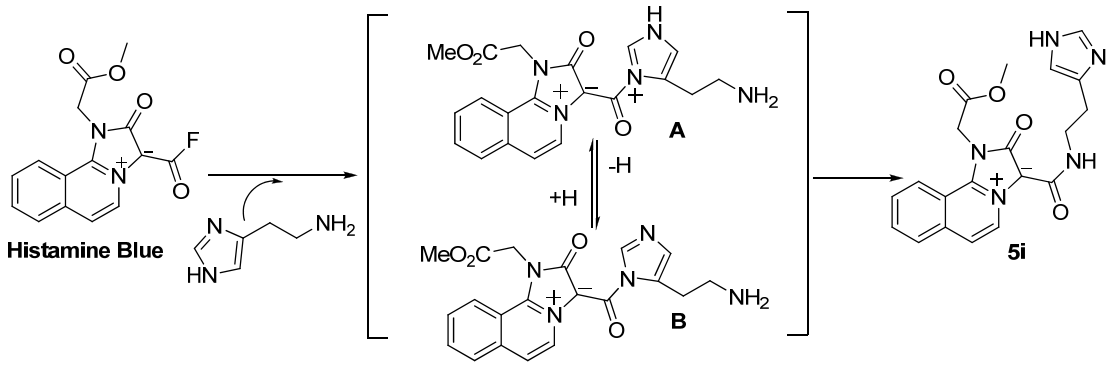


Figure S5. HPLC monitoring (350 nm) of the reactions between **Histamine Blue** and a) 2-(pyridin-2-yl)ethylamine or b) 2-phenylethylamine.

We proposed a mechanism to explain the catalytic effect observed in the reaction with histamine in which the imidazole group may act as a proton transfer moiety rather than as a nucleophilic catalyst (Scheme S3).[S7-S10]



Scheme S3. Plausible mechanism for the condensation of **Histamine Blue** with histamine.

9. Cell viability studies in RBL-2H3 basophils.

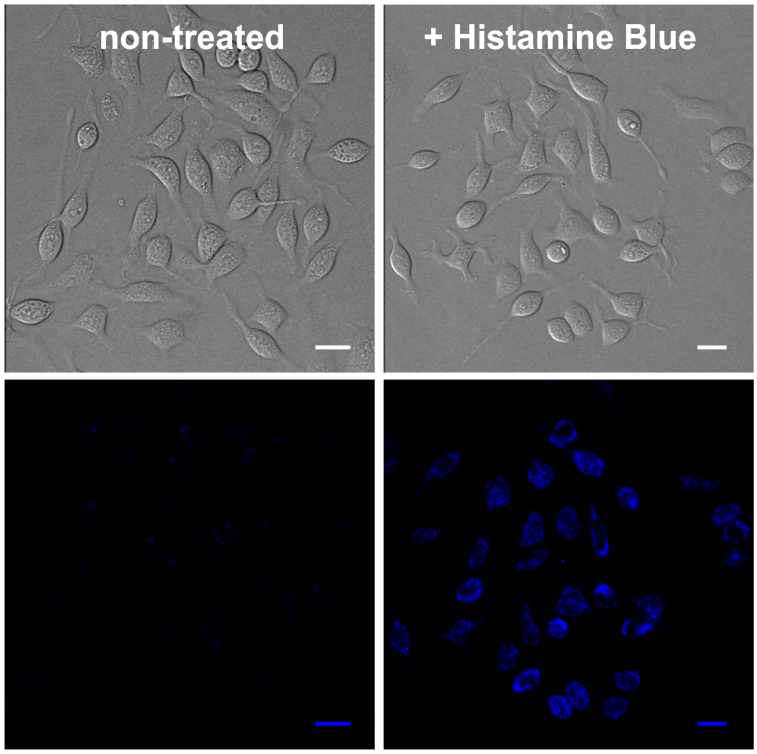


Figure S6. RBL-2H3 basophils before (left column) and after (right column) incubation with **Histamine Blue** (20 μM for 15 min at 37°C). Upper row: bright field images, lower row: **Histamine Blue** staining (DAPI channel). Scale bar: 20 μm.

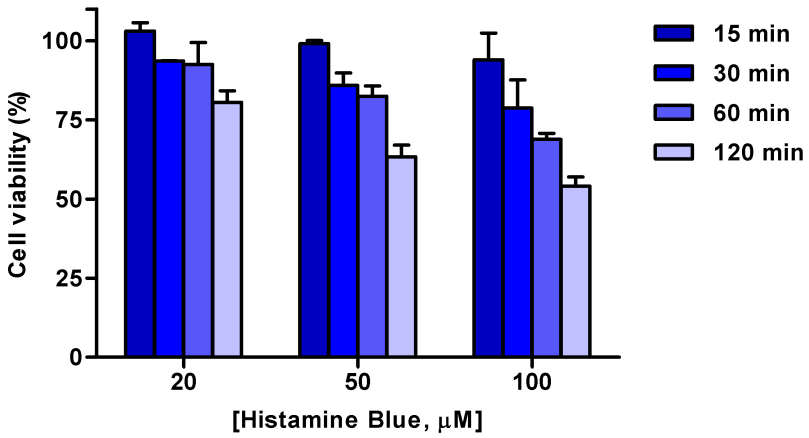


Figure S7. Cell viability of RBL-2H3 basophils after treatment with **Histamine Blue**. Cells were incubated with the dye and their cell proliferation was measured using a Cell Titer 96 Aqueous non-radioactive cell proliferation assay kit according to the manufacturer's instructions. Values represented after normalization to non-treated cells as means (n=3) and errors bars as standard deviations.

10. Titration of histamine uptake in RAW 264.7 cells.

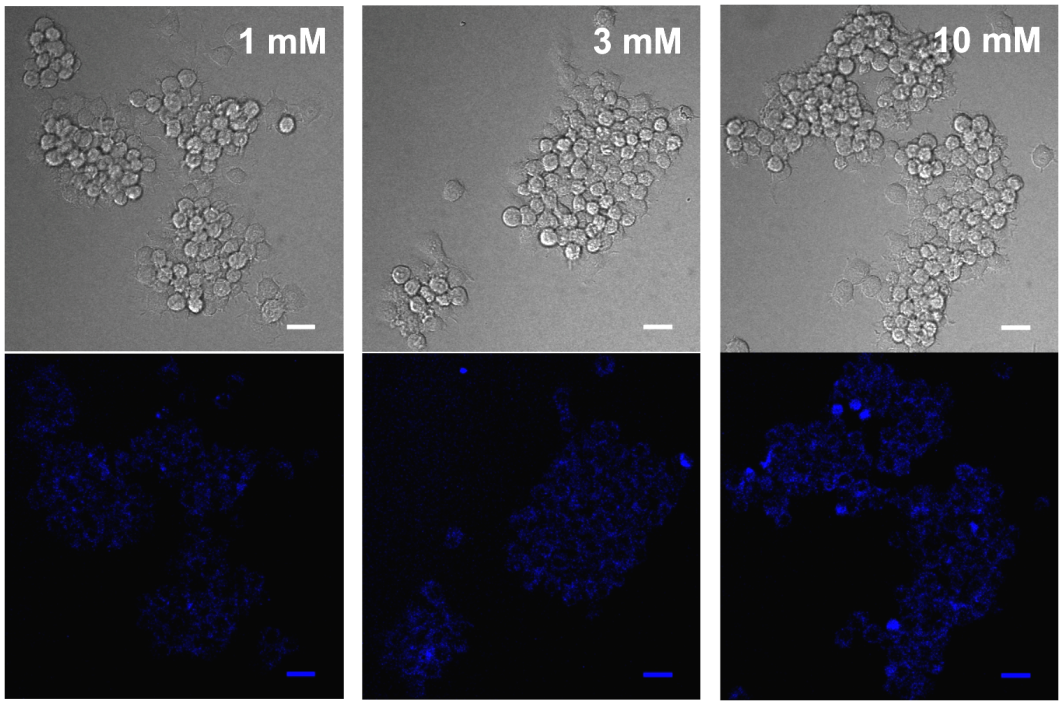


Figure S8. RAW 264.7 macrophages after incubation with different concentrations of histamine (indicated in the upper images) for 2 h at 37 ^{o}C. Subsequently, all cells were washed and treated with **Histamine Blue** (20 μM for 15 min at 37 ^{o}C). Upper row: bright field images, lower row: **Histamine Blue** staining (DAPI channel). Scale bar: 20 μm.

11. References for the Supporting Information

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