Supporting Information

BAY-6096: A potent, selective and highly water soluble adrenergic α_{2B} antagonist

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Characterization on adrenoceptor reporter cells: Adrenoceptor α1A antagonism was tested on a recombinant human α1A receptor CHO cell line, also expressing recombinant mtAeq (mitochondrial aequorin). Adrenoceptor α2A antagonism was tested on a recombinant human α2A-Gα16 receptor fusion protein CHO cell line (PerkinElmer Life Sciences), also expressing recombinant mtAeq. Adrenoceptor α2B antagonism was tested on a recombinant human α2B receptor CHO cell line (PerkinElmer Life Sciences), also expressing recombinant mtAeq. Adrenoceptor α2C antagonism was tested on a recombinant human α2C receptor HEK cell line, also expressing a chimeric G protein (Gαqi3) and Clytin. Adrenoceptor α2B antagonism was also tested in a CHO cell line expressing the human α2B receptor deletion variant (del Glu301-Glu303) and recombinant mtAeq.

Cells were cultured at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium/NUT mix F12 with L-glutamine, supplemented with 10% (v/v) inactivated fetal calf serum, 1 mM sodium pyruvate, 0.9 mM sodium bicarbonate, 50 U/ml penicillin, 50 µg/ml streptomycin, 2.5 µg/ml amphotericin B and 1 mg/ml geneticin. Cells were passaged using enzyme-free/Hank's-based cell dissociation buffer. All cell culture reagents were obtained from Invitrogen (Carlsbad, USA).
Luminescence measurements were performed on opaque 384-well microtiter plates. 2000 cells/well were plated in a volume of 25 µl and were cultured for 1 day at 30°C and 5% CO_2 in cell culture medium containing coelenterazine (α2A and α2B: 5 µg/ml; α1A and α2C: 2.5 µg/ml). Serial dilutions of the test compounds (10 µl) in Tyrode (130 mM NaCl, 5 mM KCl, 20 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 4.8 mM $NaHCO_3$ at pH 7.4) were applied to the cells. After 5 minutes norepinephrine was added to the cells (35 µl, final concentration: EC_{50} - EC_{80}) and the emitted light was measured for 50 seconds using a charge-coupled device (CCD) camera (Hamamatsu Corporation, Shizuoka, Japan) in a light tight box. Curve fitting and calculation of IC50/EC50 values was performed using GraphPad Prism Software (version 8.0, GraphPad Software Inc., San Diego, CA, USA).
Reversibility of receptor binding was tested in washout experiments. After antagonist treatment of the recombinant α2B receptor reporter cells for 5 min, the supernatant was removed. Cells were washed twice with Tyrode (35 µl). 5 min later cells were stimulated with norepinephrine.
Adrenergic receptor agonists were characterized using the reporter cell lines described above. Serial dilutions of the test compounds (10 µl) in Tyrode were applied to the cells and measurements were performed using the FLIPR® Tetra system (Molecular Devices, Sunnyvale, CA, USA).
The α2B receptor agonist **44** was identified by uHTS. **44** stimulates reporter cell lines with EC_{50} values of 3,500 nM (α2A), 46 nM (α2B), 900 nM (α2C) and >10,000 nM (α1A), respectively.

Table S1. Specificity assessment with 10 μM of compound **12** at Eurofins Panlabs Taiwan, Ltd.

Target	Significant effect
Aldose Reductase	no
ATPase, Na^+/K^+, Heart, Pig	no
Carbonic Anhydrase II	no
Cholinesterase, Acetyl, ACES	no
Cyclooxygenase COX-1	no
Cyclooxygenase COX-2	no
HMG-CoA Reductase	no
Leukotriene LTC4 Synthase	no
Lipoxygenase 15-LO	no
Monoamine Oxidase MAO-A	no
Monoamine Oxidase MAO-B	no
Nitric Oxide Synthase, Neuronal (nNOS)	no
Nitric Oxide Synthetase, Inducible (iNOS)	no
Peptidase, Angiotensin Converting Enzyme	no
Phosphodiesterase PDE3	no
Phosphodiesterase PDE4	no
Phosphodiesterase PDE5	no
Thromboxane Synthase	no
Adenosine A1	no
Adenosine A2A	no
Adenosine A3	no
Adrenergic α1A	no
Adrenergic β1	no
Adrenergic β2	no
Adrenergic β3	no
Androgen (Testosterone)	no
Angiotensin AT1	no
Angiotensin AT2	no

Bradykinin B1	no
Bradykinin B2	no
Cannabinoid CB1	no
Cannabinoid CB2	no
Dopamine D1	no
Dopamine D2L	no
Dopamine D2S	no
Dopamine D3	no
Dopamine D4.2	no
Endothelin ETA	no
Endothelin ETB	no
Estrogen Erα	no
GABAA, Chloride Channel, TBOB	no
GABAA, Flunitrazepam, Central	no
GABAB, Non-Selective	no
Glucocorticoid	no
Glutamate, AMPA	no
Glutamate, Kainate	no
Glutamate, NMDA, Agonism	no
Glutamate, NMDA, Glycine	no
Growth Hormone Secretagogue (GHS, Ghrelin)	no
Histamine H1	no
Histamine H2	no
Histamine H3	no
Insulin	no
Motilin	no
Muscarinic M1	no
Muscarinic M2	no
Muscarinic M3	no
Muscarinic M4	no
Nicotinic Acetylcholine	no
Opiate δ1 (OP1, DOP)	no

Opiate κ (OP2, KOP)	no
Opiate μ (OP3, MOP)	no
Progesterone PR-B	no
Purinergic P2X	no
Purinergic P2Y	no
Serotonin (5-Hydroxytryptamine) 5-HT1A	no
Serotonin (5-Hydroxytryptamine) 5-HT2A	no
Serotonin (5-Hydroxytryptamine) 5-HT2B	no
Serotonin (5-Hydroxytryptamine) 5-HT2C	no
Transporter, Adenosine	no
Transporter, Dopamine (DAT)	no
Transporter, GABA	no
Transporter, Norepinephrine (NET)	no
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	no
Vasopressin V1A	no

Table S2. Specificity assessment with 10 μM of BAY-6096 (**24**) at Eurofins Panlabs Taiwan, Ltd.

Target	Significant effect
Adenosine A1	no
Adenosine A2A	no
Adenosine A3	no
Adrenergic α1A	no
Adrenergic α1B	no
Adrenergic α1D	no
Adrenergic β1	no
Adrenergic β2	no
Adrenergic β3	no
Androgen (Testosterone)	no
Bradykinin B1	no
Bradykinin B2	no

Calcium Channel L-Type, Benzothiazepine	no
Calcium Channel L-Type, Dihydropyridine	no
Calcium Channel N-Type	no
Cannabinoid CB1	no
Dopamine D1	no
Dopamine D2S	no
Dopamine D3	no
Dopamine D4.2	no
Endothelin ETA	no
Endothelin ETB	no
Epidermal Growth Factor (EGF)	no
Estrogen Erα	no
GABAA, Flunitrazepam, Central	no
GABAA, Muscimol, Central	no
GABAB1A	no
Glucocorticoid	no
Glutamate, Kainate	no
Glutamate, NMDA, Agonism	no
Glutamate, NMDA, Glycine	no
Glutamate, NMDA, Phencyclidine	no
Histamine H1	no
Histamine H2	no
Histamine H3	no
Interleukin IL-1	no
Leukotriene, Cysteinyl CysLT1	no
Melatonin MT1	no
Muscarinic M1	no
Muscarinic M2	no
Muscarinic M3	no
Neuropeptide Y Y1	no
Neuropeptide Y Y2	no
Nicotinic Acetylcholine	no

Nicotinic Acetylcholine α1, Bungarotoxin	no
Opiate δ1 (OP1, DOP)	no
Opiate κ (OP2, KOP)	no
Opiate μ (OP3, MOP)	no
Phorbol Ester	no
Platelet Activating Factor (PAF)	no
Potassium Channel [KATP]	no
Potassium Channel hERG	no
Prostanoid EP4	no
Purinergic P2X	no
Purinergic P2Y	no
Rolipram	no
Serotonin (5-Hydroxytryptamine) 5-HT1A	no
Serotonin (5-Hydroxytryptamine) 5-HT2A	no
Serotonin (5-Hydroxytryptamine) 5-HT3	no
Sigma σ1	no
Sodium Channel, Site 2	no
Tachykinin NK1	no
Thyroid Hormone	no
Transporter, Dopamine (DAT)	no
Transporter, GABA	no
Transporter, Norepinephrine (NET)	no
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	no

Ames test: The assay was performed in analogy to B. N. Ames, Mutation Research **1975**, 347.

Micronucleus test: The assay was performed in analogy to the OECD test guideline 487.

hERG inhibition assay: The assay was performed in analogy to H. M. Himmel, J Pharmacol Toxicol Methods **2007**, 145.

$hNa_v1.5$ inhibition assay: The assay was performed in analogy to J. Kramer, H. M. Himmel, A. Lindqvist et al., Sci Rep 10, 5627 (**2020**). https://doi.org/10.1038/s41598-020-62344-w.

$hCa_v1.2$ inhibition assay: The assay was performed in analogy to J. Kramer, H. M. Himmel, A. Lindqvist et al., Sci Rep 10, 5627 (**2020**). https://doi.org/10.1038/s41598-020-62344-w.

$hK_{ir}2.1$ inhibition assay: The assay was performed in analogy to J. Pharmacol. Exp. Ther. **2016**, 194. https://doi.org/10.1124/jpet.116.235150.

In vivo pharmacology

All procedures conformed to European Community directives and national legislation (German law for the protection of animals) for the use of animals for scientific purposes and were approved by the competent regional authority.

Mechanistic rat model: Male Wistar rats (WiWu, Charles River, Deutschland; 270-350g body weight) were used. Rats were treated with reserpine (5 mg/kg dissolved in sesame oil, applied s.c.) at 3 consecutive days. Treatment led to depletion of catecholamines from peripheral sympathetic nerve endings and thereby to sensitization to adrenergic stimuli. Rats were anesthetized with isoflurane (5% starting dose, reduced to 2,5% during surgery and 2% during hemodynamic measurement) and placed on a warming plate. For surgery, the animals receive additional analgesia (Novalgin: 0,5 ml/kg s.c. 50 mg/kg). A tubus was inserted into the trachea for ventilation („SAR 1000 breathing pump“; 40 bpm; volumn: 4,5 ml; inspiration: 40%; volume controlled). A tip catheter was inserted in the A. femoralis to measure blood pressure (Millar, 2 French). A PE50 tube was inserted in the V. femoralis for i.v. application of the α_{2B} agonist **44** (270 µg/kg in 2 ml PBS/kg in 5 min, i.v.). A second PE50 tube was inserted in the V. jugularis for i.v. applikation of the α_{2B} antagonist (0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg in vehicle 0.5 ml/kg in 1min, vehicle: 969 g PEG400 + 60 g Glycerin + 100 g H_2O. All studies were performed with **45**, the formate of BAY-6096 (**24**). Hemodynamic parameters (BP, HR) were monitored (AD Instruments, Deutschland, LabChart 7).

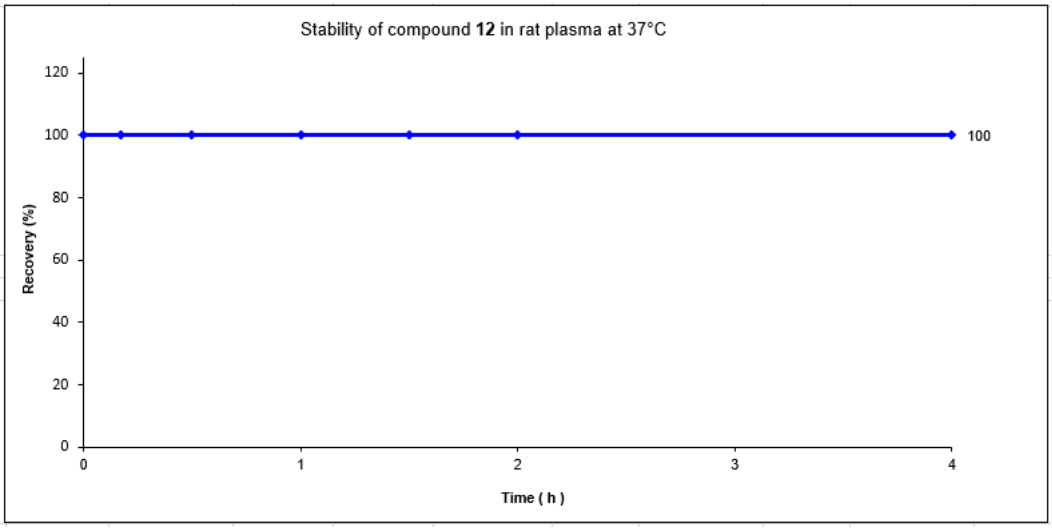
After anaesthesia and implantation of tip catheter and PE tubes, baseline BP is measured for 5 min. The animals then receive antagonist or placebo and again baseline BP is measured for 20 min. After that, **44** is infused for 5 min with continuous registration of hemodynamic parameters. Mean of measured values are determined any 6 seconds, resulting in 10 mean values any minute. BP changes after application of agonist and basal BP were calculated.

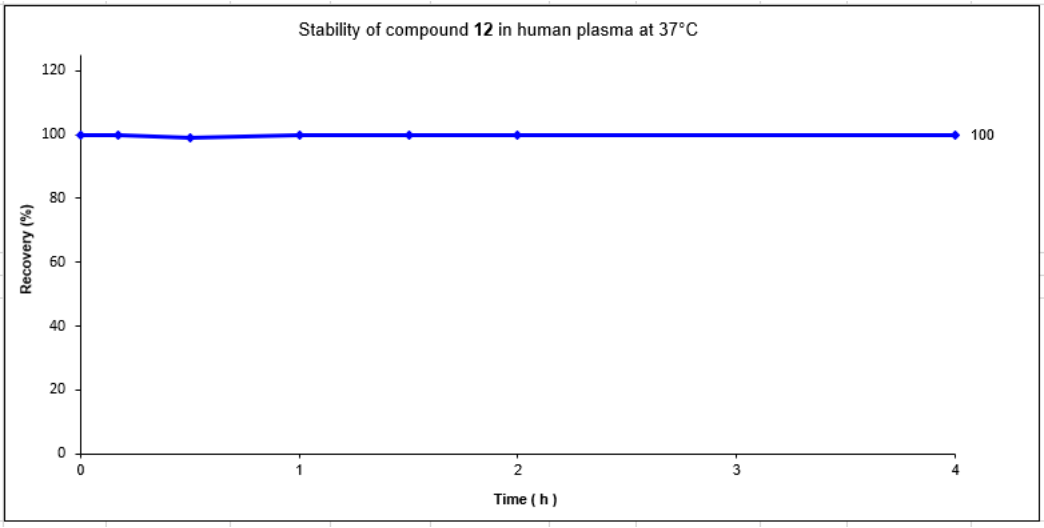
The performed mechanistic assay is a suitable method to study antagonistic action of the compound in a first approach and might bridge the gap between complex in vivo models and simple in vitro systems. It has been described in mice, that activation of α_{2B} receptors expressed in the vasculature results in BP increase [Link et al., Science, 1996]. This effect is less pronounced in the rat. Nevertheless, we were able to detect a hypertensive effect of an α_{2B} receptor agonist in rats pre-treated with reserpine. This effect was dose dependently reduced with an α_{2B} receptor antagonist.

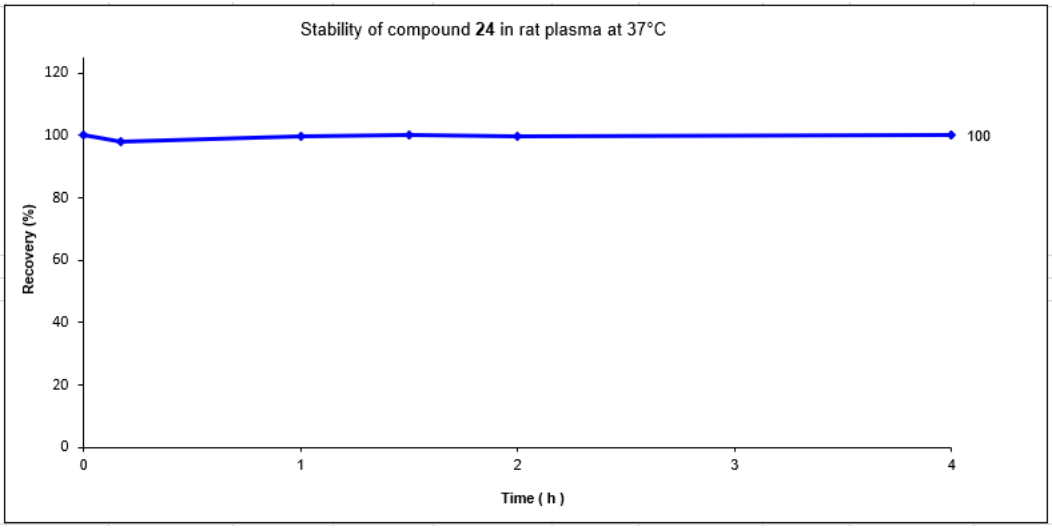
In vitro DMPK

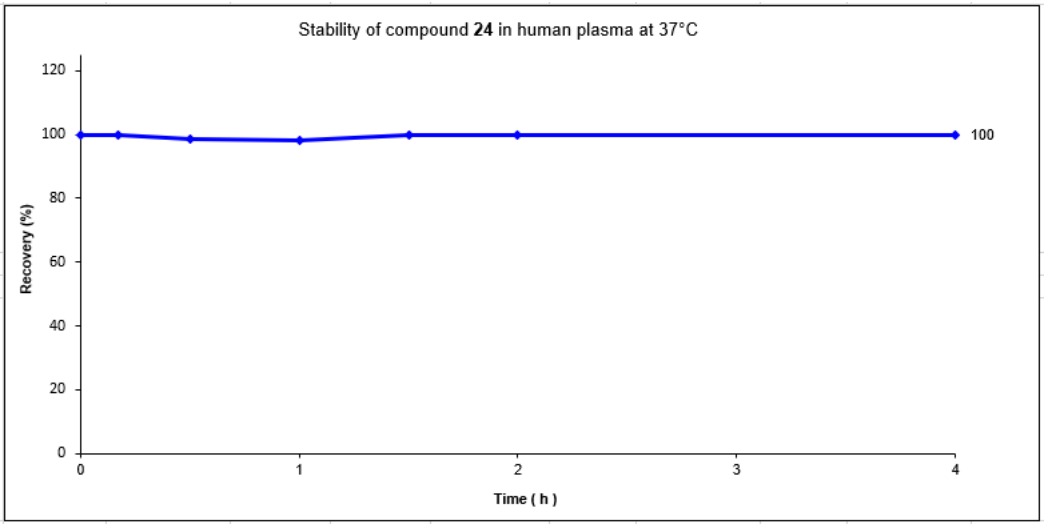
Stability in plasma: 1 mg of the test compound is dissolved in 0.5 ml acetonitrile/dimethylsulfoxide 9:1. For complete dissolution the HPLC vial is shaken and treated with ultrasound. While vertexing, 20 µl of this solution are added to 1 ml 37°C warm plasma. After 0.17, 0.5, 1, 1.5, 2 and 4 hours the enzymatic reaction is stopped. For stopping the reaction, 100 µl of the compound plasma solution are transferred to a vial containing 300 µl acetonitrile. Then the solutions are centrifuged by 5000 U/min for 10 minutes. The supernatant is analyzed by HPLC to determine the amount of the test compound. The peak areas in percentage are used for quantification.

Figure S1. Recovery of compounds **12** and **24** for 24 hours during incubation with plasma



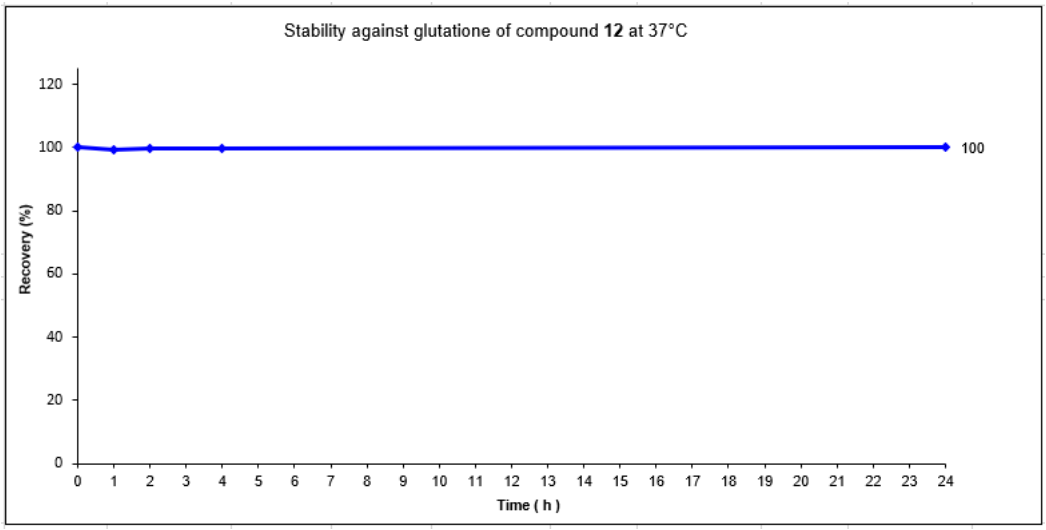






Stability against glutathione: 0.2 µmol of the test compound are dissolved in 200 µl acetonitrile. For complete dissolution the HPLC vial is shaken and treated with ultrasound. Then this solution is added to 1000 µl 37°C warm PBS buffer pH 7.4. The buffer contains 20 µmol L-glutathione. After 1, 2, 4 and 24 hours the amount of the test compound is analyzed per HPLC. The peak areas in percentage are used for quantification.

Figure S2. Recovery of compound **12** for 24 hours during incubation with glutathione



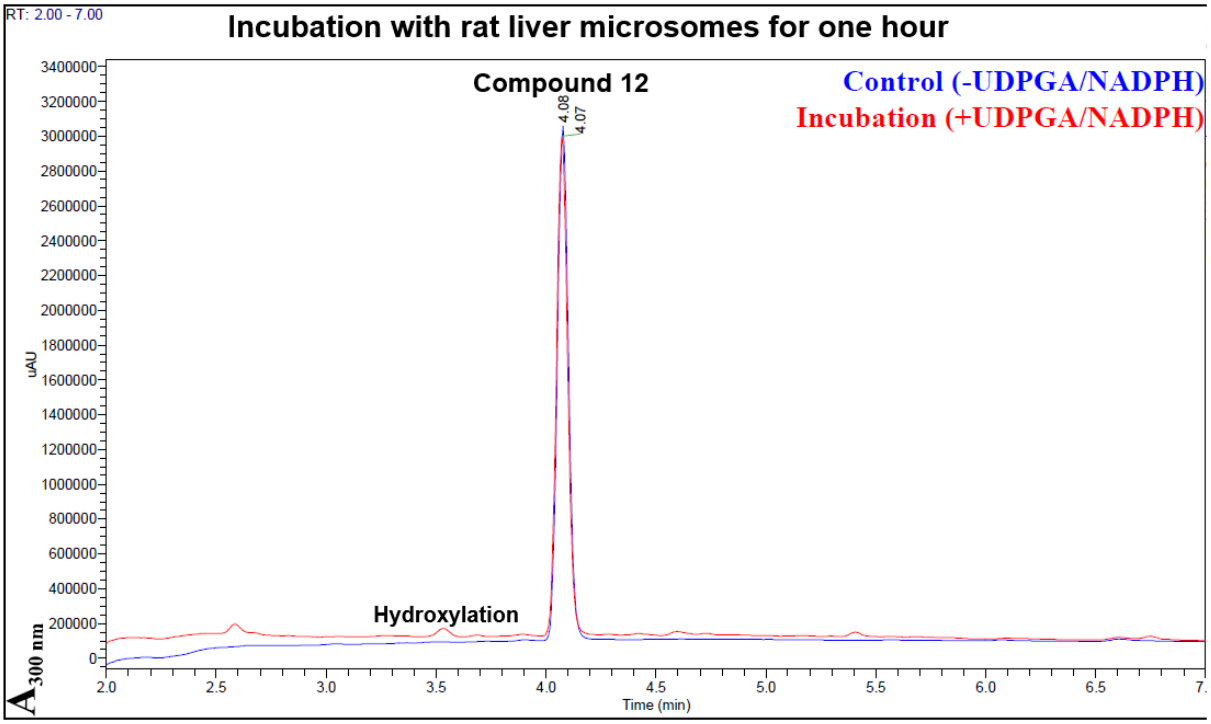
CYP inhibition assay: The ability of substances to inhibit CYP1A2, CYP2C8, CYP2C9, CYP2D6 and CYP3A4 in humans is investigated with pooled human liver microsomes or recombinantly expressed CYP3A4 (Human CYP3A4 + P450 Reductase + Cytochrome b_5 SUPERSOMES, Corning) as enzyme source in the presence of standard substrates (see below) which form CYP-isoformspecific metabolites. The inhibitory effects are investigated with six different concentrations of the test compounds (0.6, 1.3, 2.5, 5, 10 and 20 µM or 1.5, 3.1, 6.3, 12.5, 25 and 50 µM), compared with the extent of the CYP-isoform-specific metabolite formation of the standard substrates in the absence of the test compounds, and the corresponding IC_{50} values are calculated. A standard inhibitor which specifically inhibits a single CYP isoform serves as control of the results obtained. Procedure: Incubation of phenacetin, amodiaquine, diclofenac, dextromethorphan or midazolam with human liver microsomes in the presence of in each case six different concentrations of a test compound (as potential inhibitor) is carried out on a workstation (Tecan, Genesis, Crailsheim, Germany). Standard incubation mixtures comprise 1.0 mM NADP, 1.0 mM EDTA, 5.0 mM glucose 6-phosphate, glucose 6- phosphate dehydrogenase (0.5 U) and 50 mM phosphate buffer (pH 7.4) in a total volume of 200 µL. Test compounds are preferably dissolved in acetonitrile. 96-well plates are incubated with the enzyme preparation at 37°C for a defined time. The reactions are stopped by adding 100 µL of acetonitrile in which a suitable internal standard is always present. Precipitated proteins are removed by centrifugation, and the supernatants are combined and analyzed by LC-MS/MS.

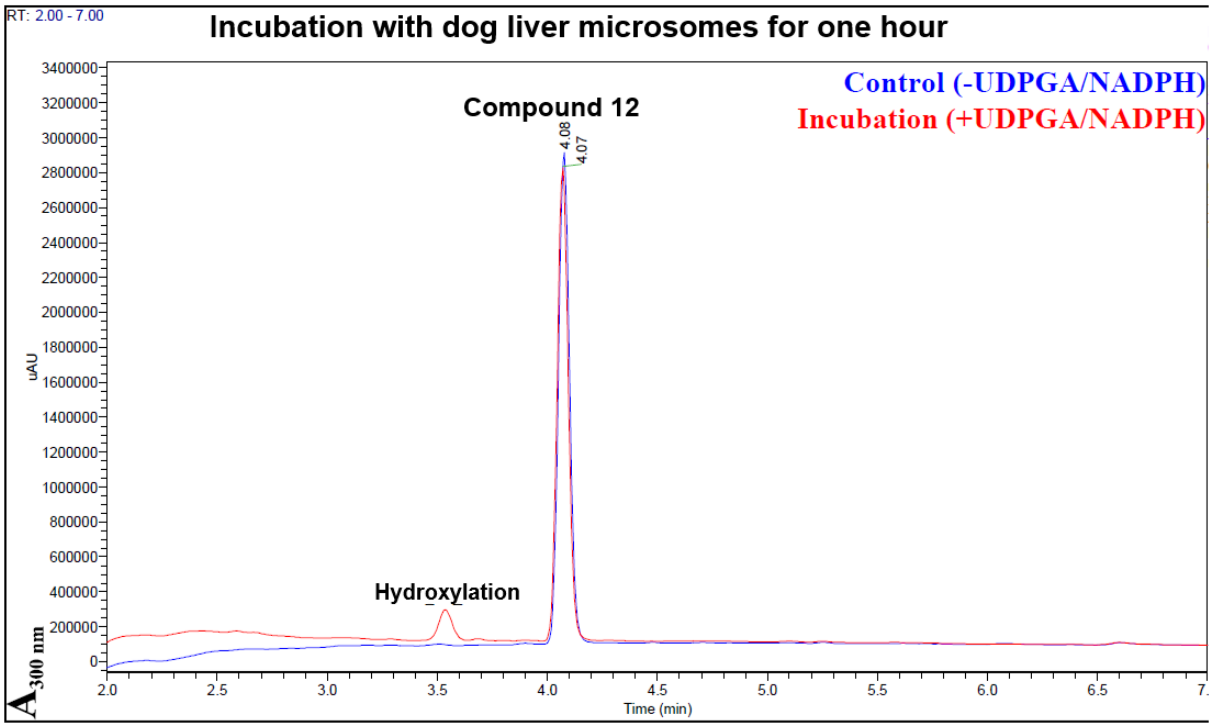
Determination of CYP induction potential: Human hepatocytes were seeded at a density of ~40.000 cells/96-well in a collagen sandwich and cultured for one day before compound treatment. Cells were treated with a 1:3 serial dilution of 8 concentrations for two consecutive days with media change every day. After 48 h of compound treatment, cells were lysed and mRNA was prepared by state of the art magnetic beads technique. Following mRNA isolation, cDNA was transcribed directly from the mRNA coated beads and further processed for qPCR. CYP relative expression levels were determined via TaqMan probes by multiplexing of CYP3A4, CYP1A2, Actin and Tubulin. Briefly, approximately 24 h after the last treatment hepatocytes were harvested for mRNA isolation. Thus, cell culture medium was removed from each well, cells were washed with 150 µL supplement free cell culture medium prior to cell lysis, cells were lysed with 100 µL lysis buffer containing proteinase K (50 $\mu g \cdot \mu L^{-1}$ (final concentration in well 30 $ng \cdot \mu L^{-1}$) and final cell lysates were stored at −80 °C. mRNA was isolated using the Dynabeads mRNA Direct Kit (Life Technologies). 150 µL of each cell lysate was mixed with 100 µg magnetic beads, incubated for a few minutes, then the supernatant was removed and beads were washed twice with Washing Buffer A (ready-to-use from kit) and twice with Washing Buffer B (ready-to-use from kit) with 200 µL and 100 µL per well, respectively. Single-stranded cDNA was prepared from mRNA with the High Capacity RNA to cDNA Kit (Life Technologies). 20 µL RT Master Mix was added to each well and transcribed for 60 min at 37 °C using the Gene Amp PCR System 9700 thermocycling program (Biometra). The RT Master Mix is comprised of 10X RT buffer, 25X deoxyNTPs, 10X Random

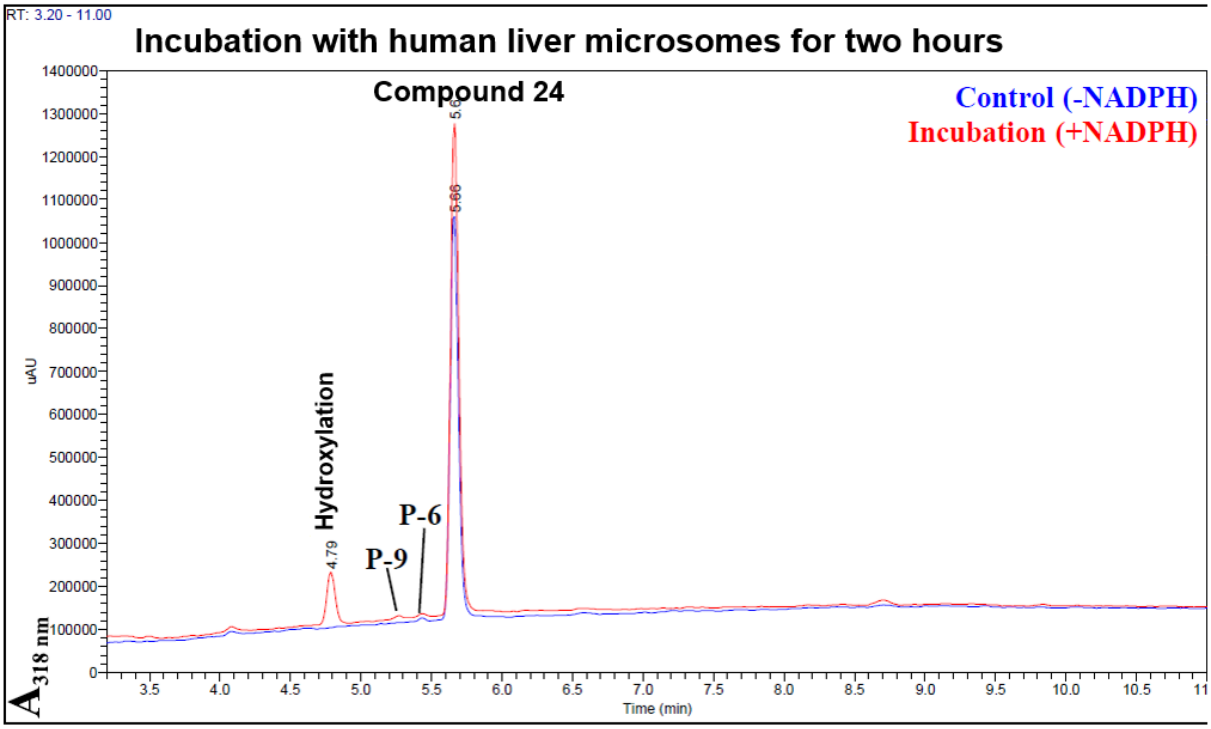
hexamers, RNase Inhibitor (20 $U \cdot \mu L^{-1}$), MultiScribe reverse transcriptase (50 $U \cdot \mu L^{-1}$) and RNase-free water. The prepared cDNA samples were stored at −80 °C prior to analysis by qRT-PCR. Quantitative RT-PCR was carried out on a QuantStudio7 Flex PCR system (Applied Biosystems) according to manufacturer`s protocol. A primer mix was prepared for each gene expression assay. A typical primer mix contained TaqMan Fast Advanced Master Mix (1X), Gene Expression Assay (1X, 900 nM forward and reverse primers) and RNase-free water and was added to the cDNA. The relative quantity of the target cDNA compared with that of the control cDNA (housekeeping genes: actin, tubulin) was determined by the ΔΔCt method. Relative quantification measures the change in mRNA expression in a test sample relative to that in a control sample (e.g. vehicle treated). In summary, CYP induction is calculated based on the ΔΔCt method and expressed as fold induction over vehicle treated control.

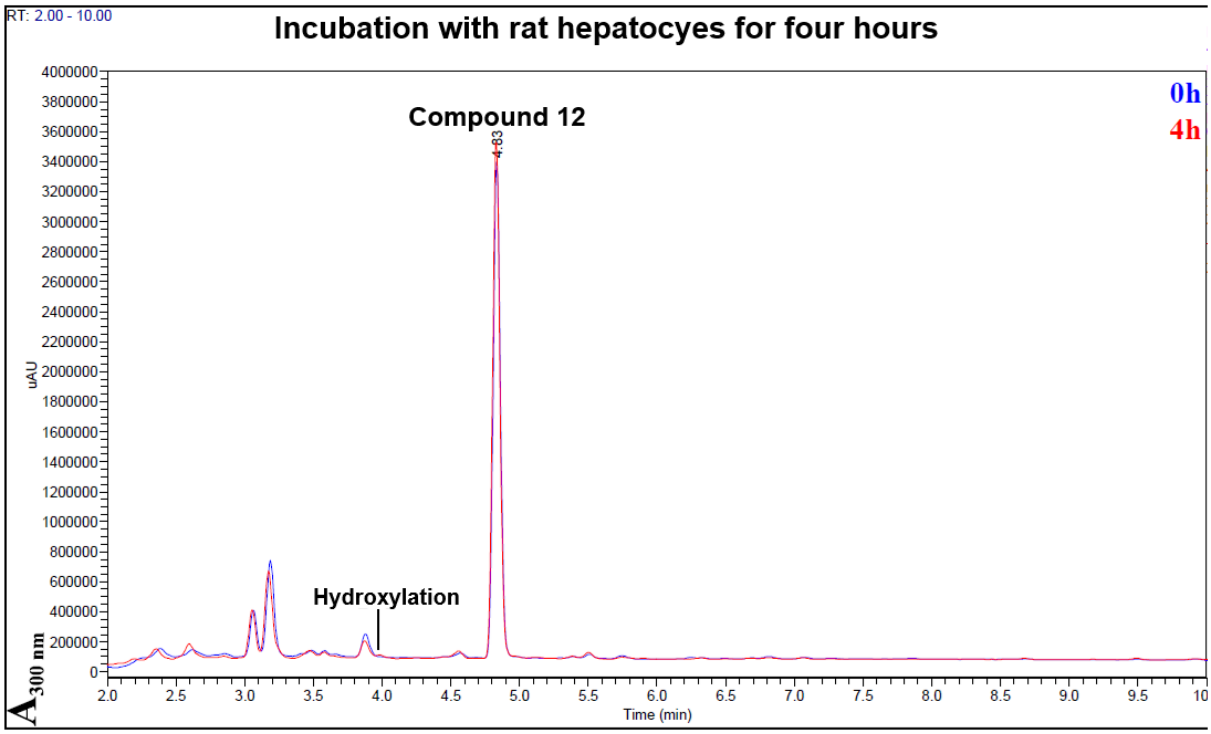
Metabolite identification assay: Hepatocytes from rat were prepared in-house at Bayer AG according to standard procedures. Cryopreserved human, dog and cynomolgus monkey hepatocytes as well as human liver microsomes were purchased from commercial suppliers. Hepatocytes were incubated with 10 µM test compound in Williams' medium E in suspension cultures ($1 \cdot 10^6$ cells mL^{-1}) at 37 °C. Aliquots were removed from the incubation mixture at the beginning (0 hours) and after 1, 2, and 4 hours. Rat, dog or human liver microsomes were incubated in 50 mM potassium phosphate buffer pH 7.4 containing 1.0 mM EDTA in the absence and presence of cofactors NADPH/UDPGA. Metabolite patterns were analyzed using high resolution LC/MS/MS (Agilent 1290 HPLC, Thermo Orbitrap Fusion high resolution mass spectrometer) in ddMS2 mode.

Figure S3. UV traces of compounds **12** and **24** after incubation with microsomes or hepatocytes




RT: 2.00 - 7.00
Incubation with dog liver microsomes for one hour
Control (-UDPGA/NADPH)
Incubation (+UDPGA/NADPH)
Compound 12
4.08
4.07
Hydroxylation
uAU
A300 nm
Time (min)


RT: 3.20 - 11.00
Incubation with human liver microsomes for two hours
Compound 24
Control (-NADPH)
Incubation (+NADPH)
5.66
4.79
Hydroxylation
P-6
P-9
uAU
A318 nm
Time (min)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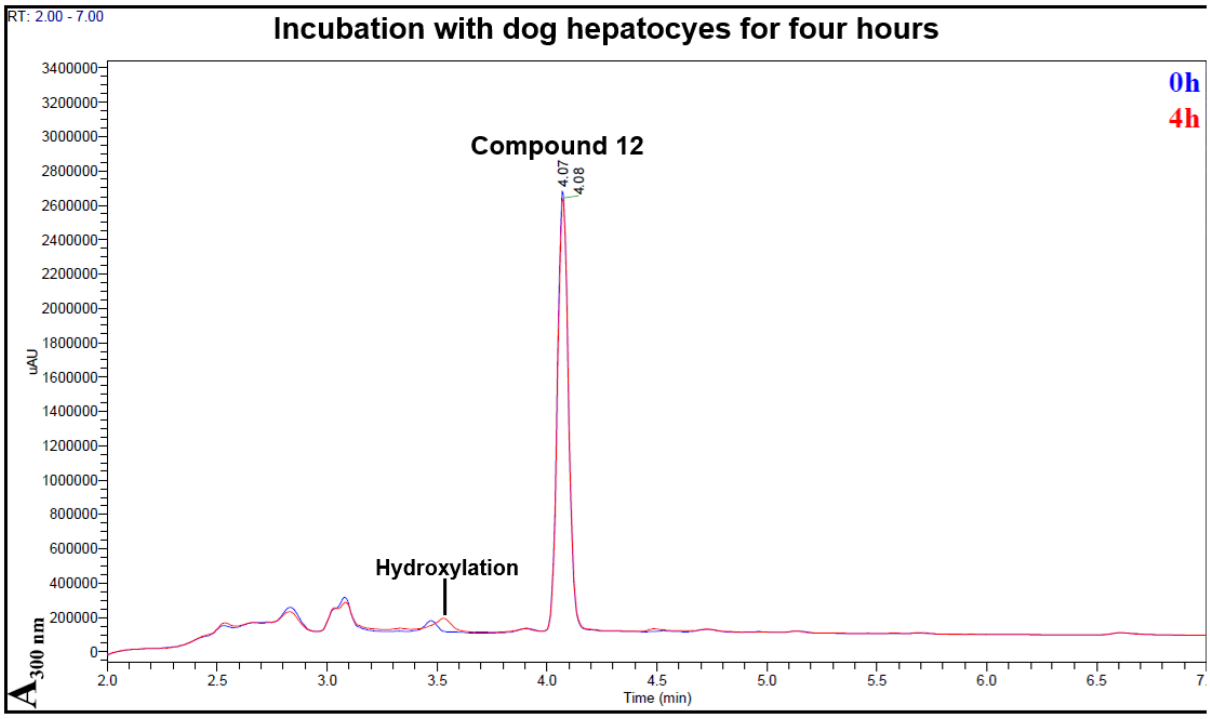
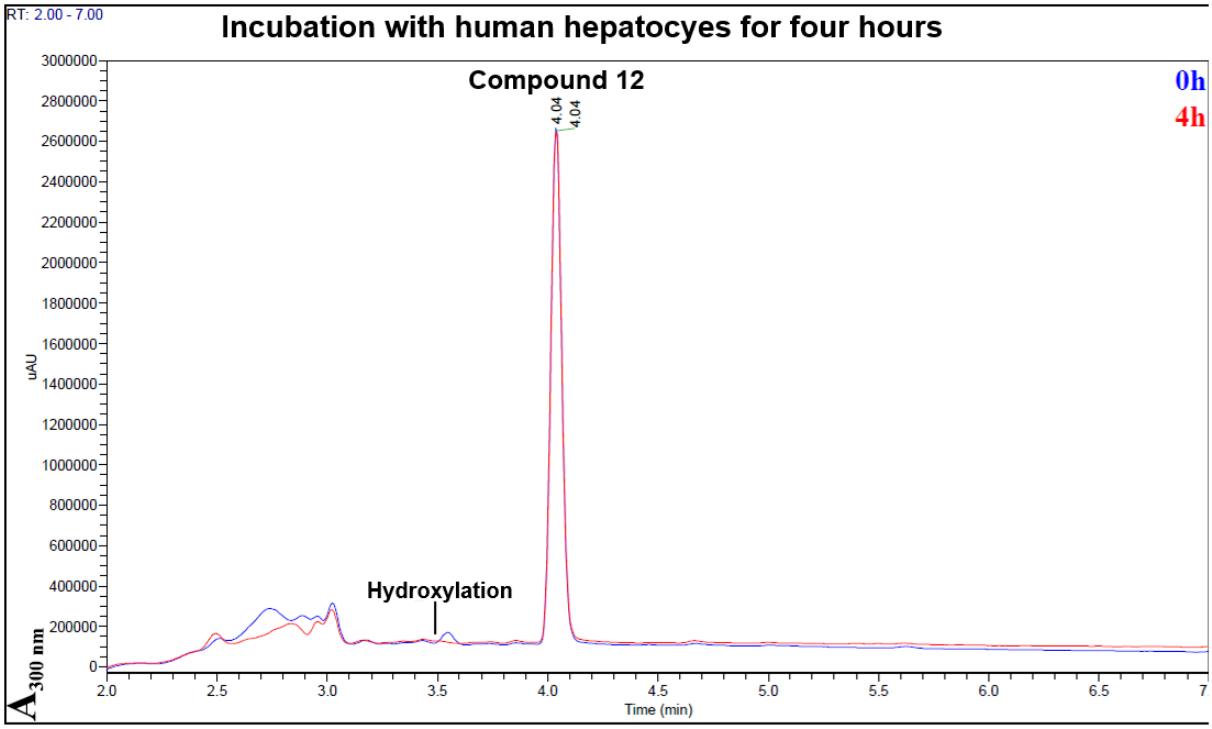
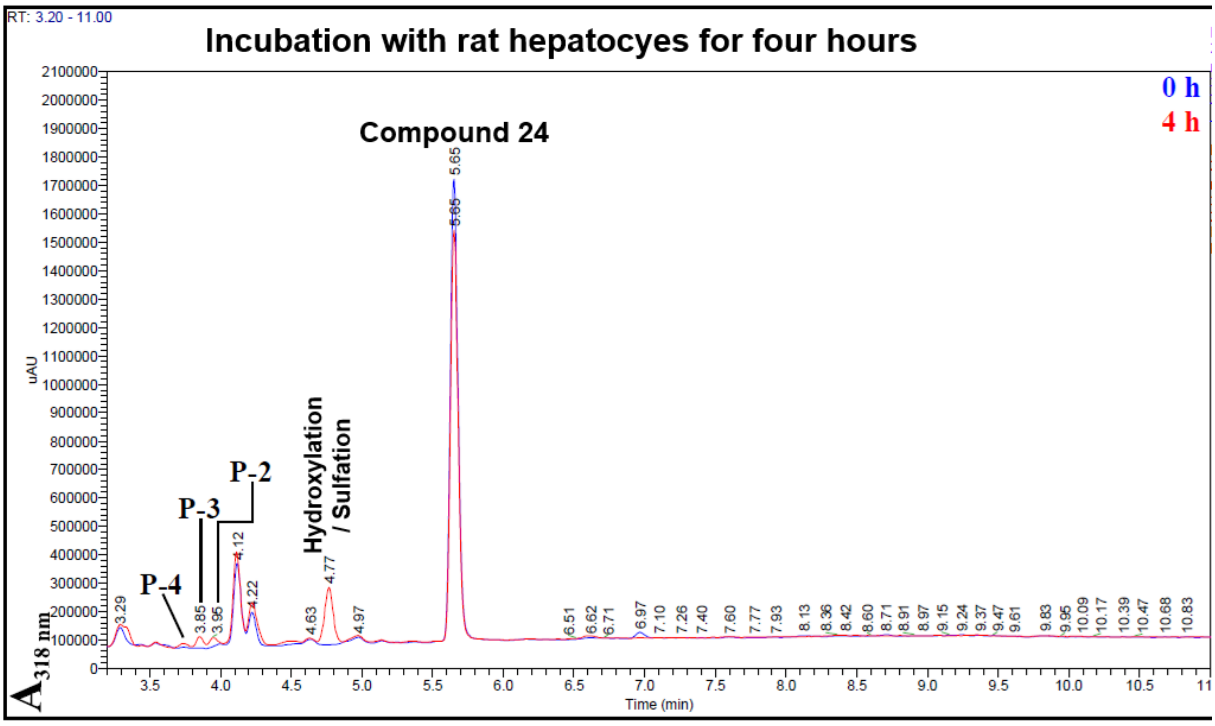
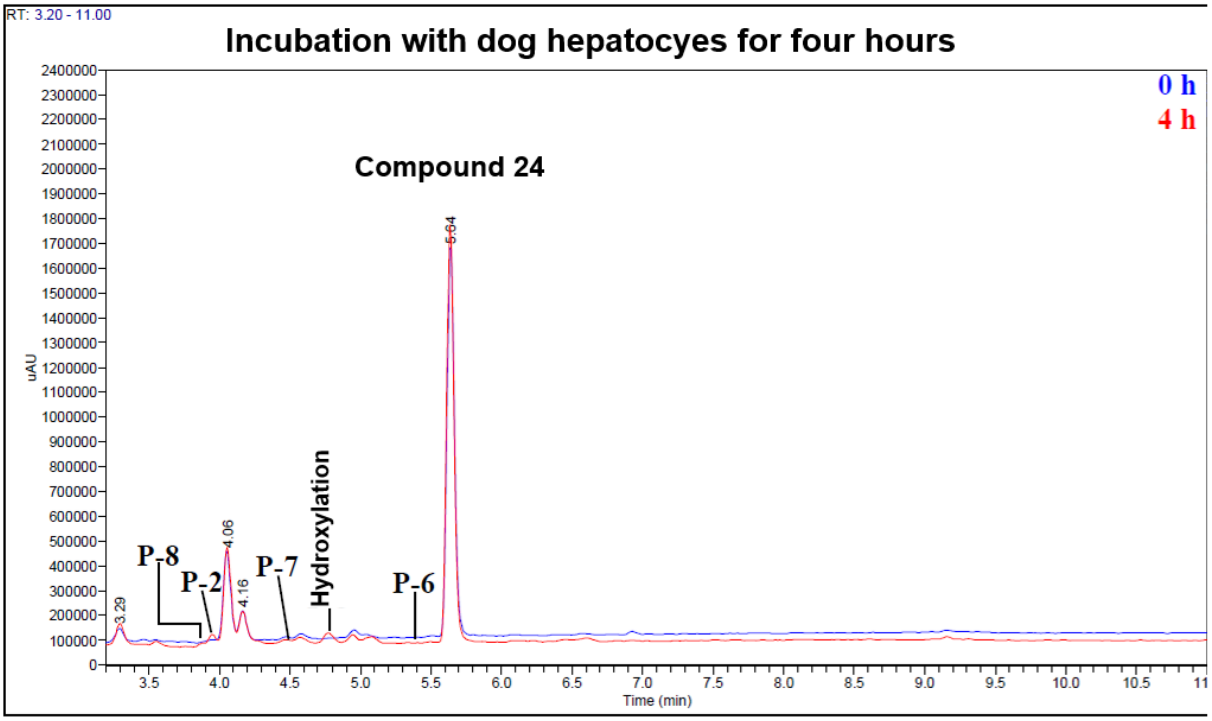
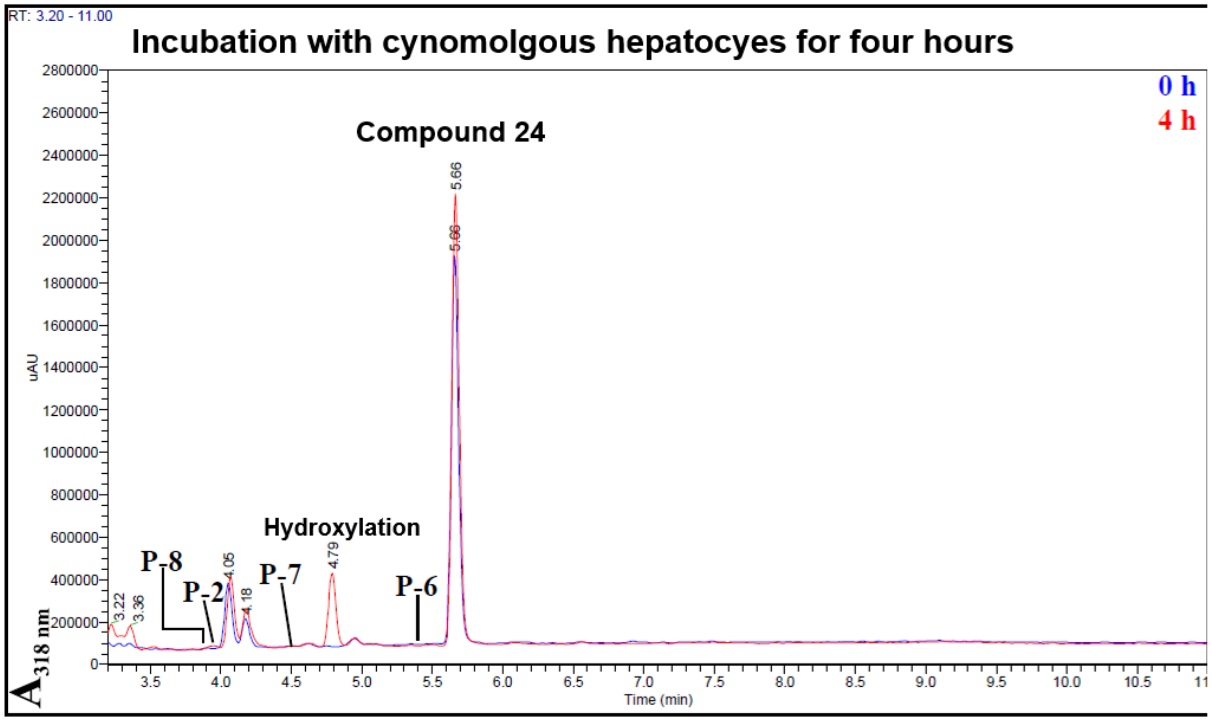
Incubation with rat hepatocyes for four hours
Compound 12
0h
4h
4.83
Hydroxylation
uAU
A300 nm
Time (min)

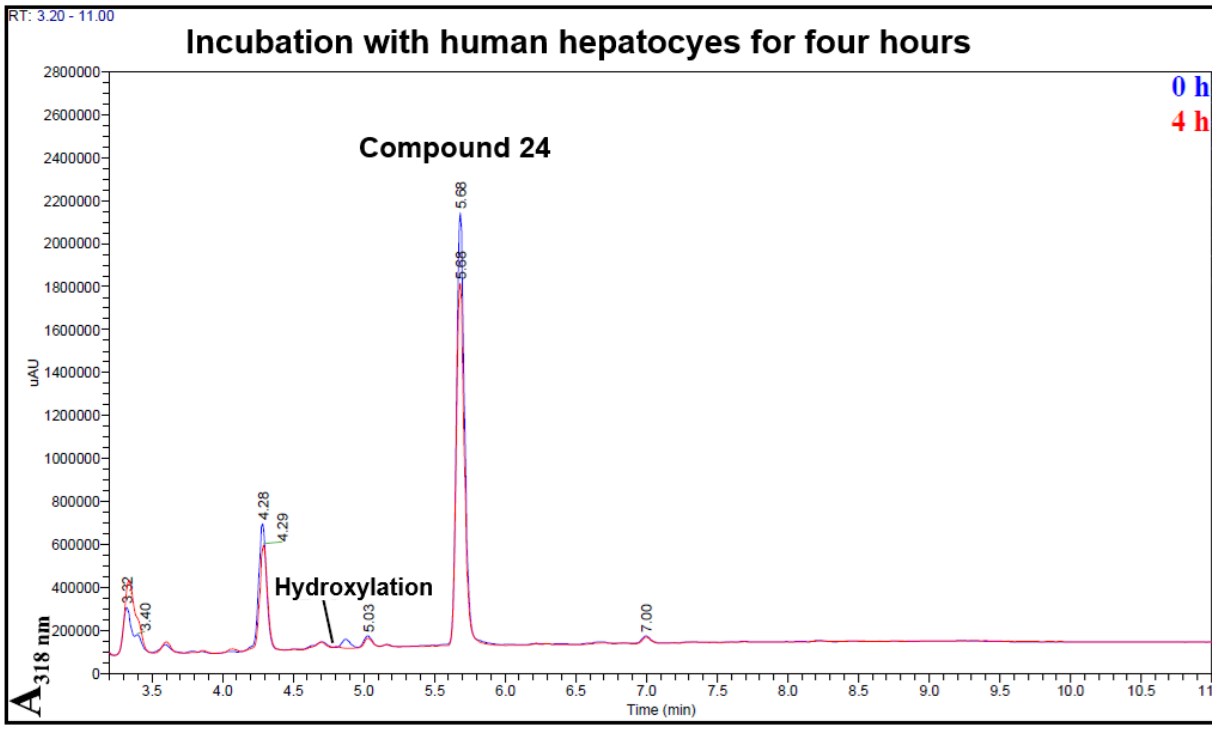

RT: 2.00 - 7.00
Incubation with dog hepatocyes for four hours
0h
4h
Compound 12
4.07
4.08
Hydroxylation
uAU
A300 nm
Time (min)


RT: 2.00 - 7.00
Incubation with human hepatocyes for four hours
0h
4h
Compound 12
4.04
4.04
Hydroxylation
uAU
A300 nm
Time (min)


RT: 3.20 - 11.00
Incubation with rat hepatocyes for four hours
0 h
4 h
Compound 24
P-4
P-3
P-2
Hydroxylation
/ Sulfation
A318 nm
uAU
Time (min)


RT: 3.20 - 11.00
Incubation with dog hepatocyes for four hours
0 h
4 h
Compound 24
P-8
P-2
P-7
Hydroxylation
P-6
uAU
Time (min)





Determination of plasma protein binding: Plasma protein binding was assessed by means of equilibrium dialysis, using the 96-well HT equilibrium dialysis device by HTDialysis LLC (Gales Ferry, USA) and dialysis membrane strips with a molecular weight cutoff of 12–14 kDa. Membrane strips were hydrated using *aqua demineralisata* and 30% EtOH according to the manufacturers protocol. The dialysis block was prepared by adding 150 µL of 20 mM PBS buffer to the receiver side of the dialysis chamber. Next, 150 µL of plasma from selected species (pooled from 3 individuals), either undiluted or diluted 1:10 with 20 mM PBS buffer, spiked with defined concentrations of the respective test compound, was filled into the donor compartments of the dialysis block. Incubations were performed for 6 h at 37 °C in a 7% CO_2

atmosphere. After the incubation, 100 µL from each side of the dialysis chamber were used for determination of the compound concentrations in the donor and receiver compartments. For measuring concentrations of compounds, LC-MS/MS analysis was employed. The effect of the plasma dilution on the plasma protein binding was corrected as described in the literature,[3] when diluted plasma was used for the experiment.

Permeability in Caco-2 cells: This test is designed to determine permeability values (Papp) of selected compounds using Caco-2 cells cultured for 14 days on 24-well plates. The test could be done by a Tecan robot or manually. Caco-2 cells are used as an in vitro model for screening compounds for their potential of intestinal absorption in mammalians. Furthermore, this assay allows to discover active transport processes. Method: The integrity of the cell monolayer is tested by measuring TEER values. Test compounds are dissolved in DMSO and then diluted to a final concentration of 2 µM. The proportion of remaining organic solvent is not higher than 1%. The flux is investigated for both directions by adding the solution to the apical or basal compartment. Cover plates are incubated for 2 h at 37 °C. Samples are measured by LC-MS/MS.

Metabolic stability in hepatocytes: Metabolic stabilities of NCEs in hepatocytes are determined by incubating the compounds at low concentrations (preferably below 1 µM) and at low cell numbers (preferably at $1 \cdot 10^6$ cells mL^{-1}) to ensure linear kinetics as good as possible. 7 timepoints from the incubation mixture are drawn for analysis to define the half-life of the compound. From that half-life, different clearance values and an Fmax value are calculated for the compound. The CL and Fmax values reflect Phase I and II metabolism in hepatocytes. To minimize the influence of organic solvents in the incubation mixture their content is limited to max. 1% for ACN or max. 0.2% for DMSO. For all species and strains, the cell number of hepatocytes is supposed to be $1.1 \cdot 10^8$ cells/g liver.

In vivo PK

i.v. Pharmacokinetics in Wistar Rats: On the day before administration of the substance, a catheter for obtaining blood is implanted in the jugular vein of the experimental animals (male Wistar rats, body weight 200-250 g) under Isofluran® anesthesia. On the day of the experiment, a defined dose of the test substance is administered as solution into the tail vein (short term infusion, duration of administration 15 min). The formulation in rats is plasma / dimethyl sulfoxide (99/1). Blood samples (8-12 time points) are taken through the catheter sequentially over the course of 24 h after administration of the substance. Plasma is obtained by centrifuging the samples in EDTA tubes. Trichloroacetic acid is added to a defined plasma volume per time point to precipitate proteins. After centrifugation, test substance and, where appropriate, known cleavage products of the test substance in the supernatant are determined quantitatively using a suitable LC/MS-MS method. The measured plasma concentrations are used to calculate pharmacokinetic parameters of the test substance and of the active ingredient compound liberated therefrom, such as AUC, C_{max}, $t_{1/2}$ (half-life) and CL (clearance).

i.v. Pharmacokinetics in Dogs: To investigate the pharmacokinetic properties of the substances, animals (e.g. dogs) can be injected with the respective substances by infusion (15 min). The substances are preferably formulated in 0.9% sodium chloride solution, polyethylene glycol / ethanol / water in a ratio of 50/10/40 (other suitable formulations are also possible).

Blood samples can be taken from the animals via a catheter or venipuncture and collected in tubes containing anticoagulants (e.g. lithium heparinate or potassium EDTA). Blood samples are taken from the test animals at the following times: 0.033, 0.083, 0.167, 0.25, 0.283, 0.333, 0.5, 0.75, 1, 2, 3, 5, 7, 24 hours after administration of the substance. It is also possible to take fewer, further or later times. The blood samples are centrifuged to obtain plasma. The supernatant (plasma) is removed and either processed further directly or frozen for later sample processing. For sample preparation, 50 µL plasma is mixed with 800 µL trichloroacetic acid (also containing the internal standard ISTD for later analytical determination) and then left to stand for 5 minutes at room temperature. The mixture is then centrifuged for 10 minutes at 16,000 g. The supernatant is removed. The samples are then analyzed using LC-MS / MS (e.g. liquid chromatography with a Gemini 5µM C18 110A 50x3mm (or 150x3mm) column from

Phenomenex; mass spectrometry with an API 5500 or API 6500; SCIEX, Canada) to determine the concentration of the substance in the individual samples examined.

The pharmacokinetic parameters are calculated by non-compartmental analysis (NCA). The algorithms for calculating the parameters are based on rules that are published in general textbooks on pharmacokinetics (e.g. Rowland and Tozer, Clinical Pharmacokinetics and Pharmacodynamics, ISBN 978-0-7817-5009-7).

The primary pharmacokinetic parameters clearance (CL) and volume of distribution (Vss) can be calculated as follows:

Table S3. Definition of pharmacokinetic parameters

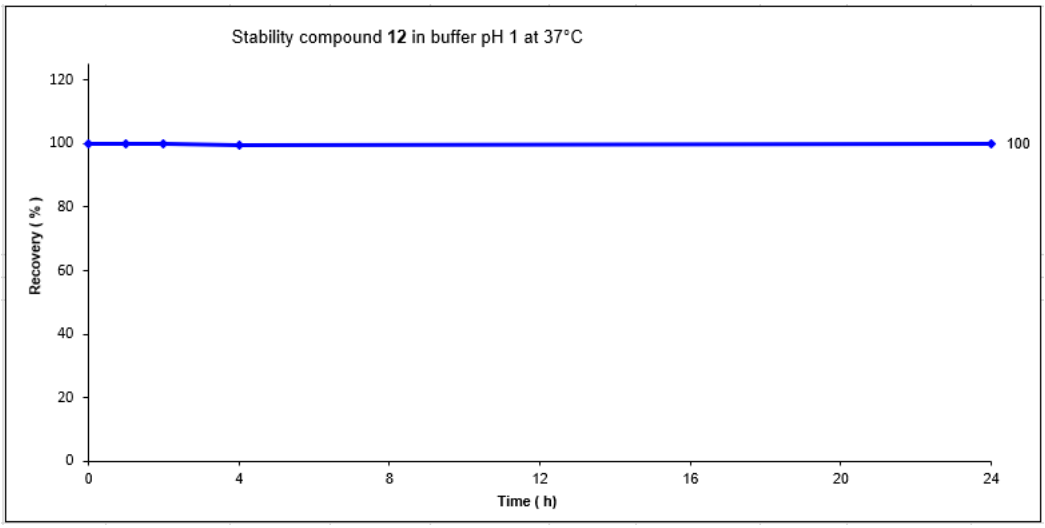
Parameter	**Formula**
CL (Plasma Clearance)	CL = Dose / AUC (AUC = Area under the curve)
Vss	Vss = CL * MRTiv
MRTiv	MRTiv = AUMC/AUC $- t_{infusion}*0.5$
AUMC	AUMC = AUMC(0-t_{last}) + $t_{last}*C_{last,calculated}/\lambda_z + C_{last,calculated}/\lambda_z^2$
λ_z	Rate constant for the terminal phase; is calculated from the logarithmic-linear regression of unweighted data from the terminal phase with data points above the detection limit
AUC	AUC = AUC(0-tlast) + $C_{last,calculated}/\lambda_z$

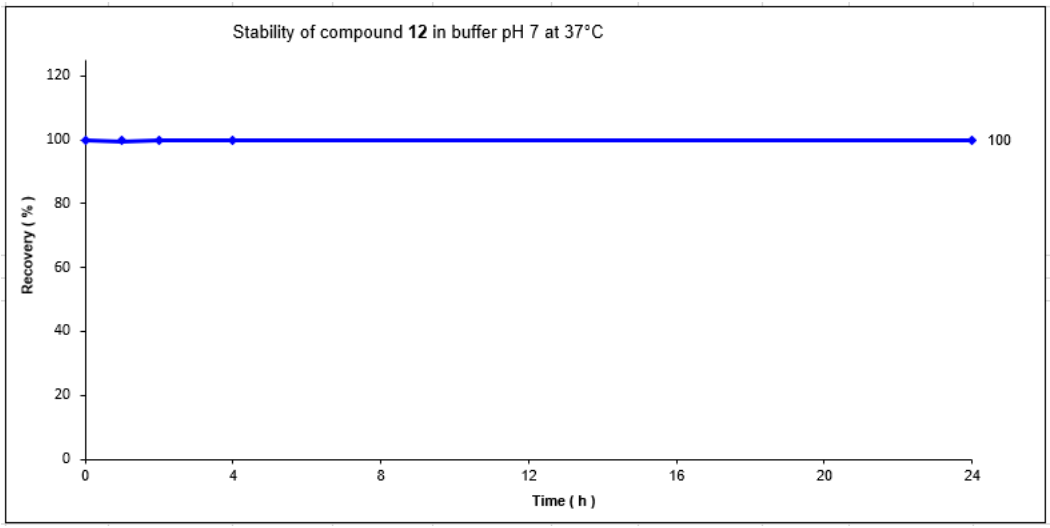
Chemistry

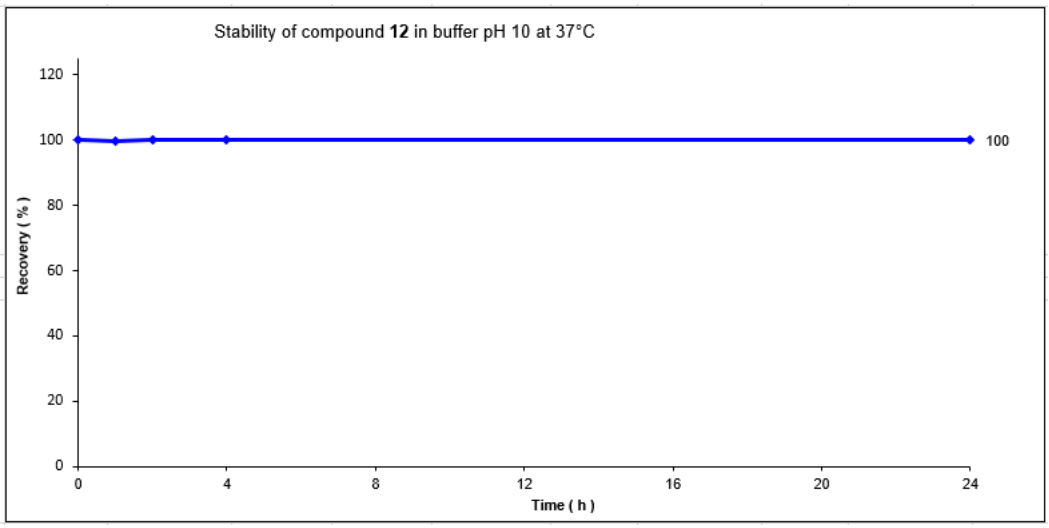
Physicochemical characterization

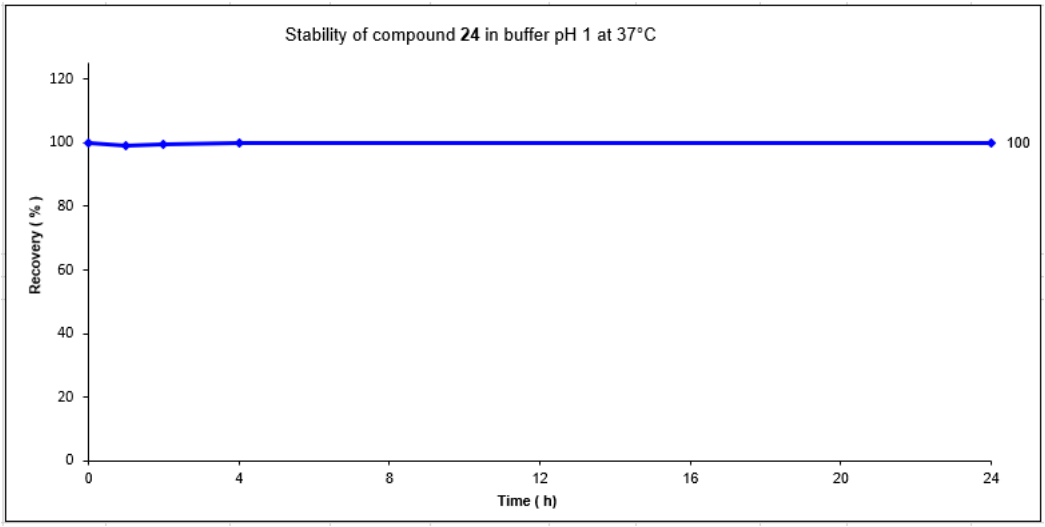
Hydrolytic stability: 0.15 mg of the test compound are dissolved in 0.1 ml dimethylsulfoxide and 0.4 ml acetonitrile. For complete dissolution the HPLC vial with the sample solution is shaken and treated with ultrasound. Then 1.0 ml of the respective buffer solution is added, and the sample is vortexed. The sample solution is analyzed by HPLC to determine the amount of the test compound at a particular time over a period of 24 h at 37 °C. The peak areas in percentage are used for quantification.

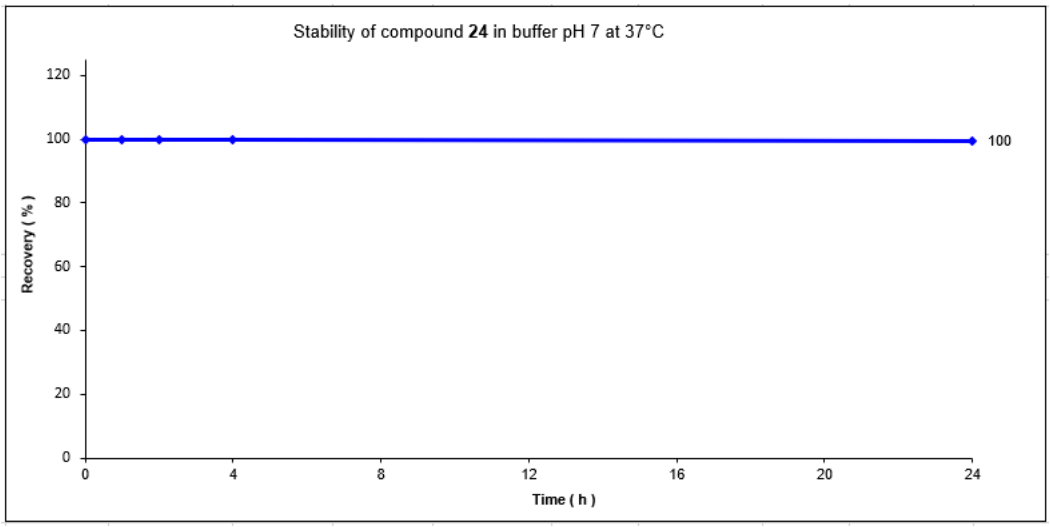
Figure S4. Recovery of compounds **12** and **24** for 24 hours during incubation with pH buffer

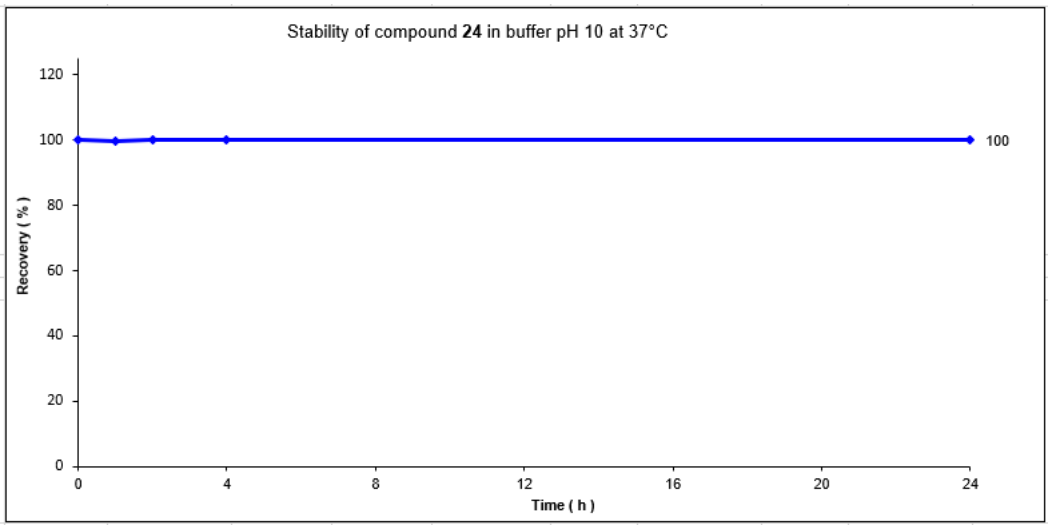












Additionally, hydrolytic stability of **24** was tested over 7 days. In this assay, solutions of the compound (0.5 mg/mL drug substance in 50% 0.1 M HCl (pH 1) or buffer pH 7 or buffer pH 10 and 50% methanol) were stored for 7 days at room temperature and analyzed per HPLC (Agilent 1290 Infinity II LC system; Column: Zorbax SB-CN RRHD 1.8 µm 50 x 1 mm; Eluent A: Buffer pH 7.2 (10 mM), Eluent B: Acetonitrile; Gradient: 0.0 min 95% A → 3.0 min 70% A → 7.0 min 20% A → 9.0 min 20% A. Oven: 40 °C; Flow: 0.60

ml/min; UV detection: 220 nm). The compound showed hydrolytic stability at all tested pH values over a period of 7 days at RT.

Table S4. Hydrolytic stability of BAY-6096

Conditions	BAY-6096 [peak area %]	Sum of all organic impurities, [peak area %]	Largest degradation/ secondary component product(s) [peak area %]
0.1 M HCl / pH 1 Initial	100.0	0.0	not detected
0.1 M HCl / pH 1 1 week, RT	100.0	0.0	not detected
Buffer pH 7 Initial	100.0	0.0	not detected
Buffer pH 7 1 week, RT	100.0	0.0	not detected
Buffer pH 10 Initial	100.0	0.0	not detected
Buffer pH 10 1 week, RT	100.0	0.0	not detected

Solubility pH 7: The assay determines the saturation solubility from solids at pH 7. Approximately 10 mg of each substance is weighed exactly into a 2 ml Eppendorf tube. 0.5 ml of buffer is pipetted into the sample. This sample solution or sample suspension is shaken for 24 h at RT and 1400 rpm. For the calibration, 0.5 - 1.0 mg are weighed exactly into a 2 ml Eppendorf tube. A stock solution with a concentration of c = 600 µg / ml is prepared from this initial weight using DMSO. Further dilution is carried out manually. For this purpose, 3 calibration solutions are made from the stock solution. After shaking the sample solutions / sample suspensions, 2 × 230 µl of the supernatant are transferred into centrifuge tubes and centrifuged at 42,000 rpm for 30 min. After centrifugation, 180 µl of the supernatant are removed and combined. Further dilution is carried out manually. For the 1:10 and 1:100 dilutions, the buffer is preferably used otherwise DMSO. The 1:100 and 1:500 dilutions are chromatographed. If the solubility is poor, the 1:10 dilution is also chromatographed.

Solubility of crystalline BAY-6096 (24): A solution or suspension of the drug substance in 0.9% NaCl solution was stirred at 25°C for 24 h ± 4 h, filtrated and analyzed via HPLC using a calibration curve (external standard).

Feasibility of sterilization of BAY-6096 (24): Feasibility of sterilization was tested with a 0.1 mg/mL solution of BAY-6096 in 0.9% NaCl solution (pH 7.4). The drug content was determined by HPLC in the untreated formulation, after sterile filtration with a 0.22 µm PVDF filter (Millex-GV, Merck Millipore) and after heat steam sterilization (121°C, 15 min). Neither adsorption to the sterile filter nor degradation upon heat steam sterilization were observed.

Table S5. Stability of BAY-6096 during sterilization

Process step	Largest degr. product [peak area %]	BAY-6096 [peak area %]	Content [% of theoretical]
Start	n.d.	99.91	101.30
Sterile filtration	n.d.	99.92	100.90
Heat steam sterilization	n.d.	99.91	100.90

General Methods

All commercial reagents and catalysts were used as provided by the commercial supplier without purification. Solvents for synthesis, extraction, and chromatography were of reagent grade and used as received. Moisture-sensitive reactions were carried out under an atmosphere of argon, and anhydrous solvents were used as provided by the commercial supplier. Reaction progress was monitored by HPLC, LC/MS or thin layer chromatography. Crude products were immediately purified using preparative reversed-phase HPLC methodology with UV detection or flash chromatography on silica gel. The fractions obtained were concentrated in vacuo to remove organic volatiles. Unless otherwise indicated, all compounds have greater than 95% purity.

Analytical Methods

NMR Spectroscopy: ^{1}H NMR and ^{13}C NMR spectra were recorded in solvents indicated below at RT with Bruker Avance spectrometers operating at 400, 500 or 600 MHz for ^{1}H NMR; at 126 MHz for ^{13}C NMR. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard. The descriptions of the coupling patterns of ^{1}H NMR signals are based on the optical appearance of the signals and do not necessarily reflect the physically correct interpretation. In general, the chemical shift information refers to the center of the signal. In the case of multiplets, intervals are given. Spin multiplicities are reported as s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet.

LC/MS-Method 1: Instrument: Waters ACQUITY SQD UPLC System; Column: Waters Acquity UPLC HSS T3 1.8 µm 50 x 1 mm; Eluent A: 1 l Water + 0.25 ml 99%ige Formic acid, Eluent B: 1 l Acetonitrile + 0.25 ml 99%ige Formic acid; Gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A. Oven: 50°C; Flow: 0.40 ml/min; UV-Detection: 210 nm.

LC/MS-Method 2: System MS: Thermo Scientific FT-MS; System UHPLC+: Thermo Scientific UltiMate 3000; Column: Waters, HSST3, 2.1 x 75 mm, C18 1.8 µm; Eluent A: 1 l Water + 0.01% Formic acid; Eluent B: 1 l Acetonitrile + 0.01% Formic acid; Gradient: 0.0 min 10% B → 2.5 min 95% B → 3.5 min 95% B; Oven: 50°C; Flow: 0.90 ml/min; UV-Detection: 210 nm/ Optimum Integration Path 210-300 nm.

LC/MS-Method 3: Instrument: Waters ACQUITY SQD UPLC System; Column: Waters Acquity UPLC HSS T3 1.8 µm 50 x 1 mm; Eluent A: 1 l Water + 0.25 ml 99%ige Formic acid, Eluent B: 1 l Acetonitrile + 0.25 ml 99%ige Formic acid; Gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A Oven: 50°C; Flow: 0.35 ml/min; UV-Detection: 210 nm.

LC/MS-Method 4: Instrument: Waters Single Quad MS System; Instrument Waters UPLC Acquity; Column: Waters BEH C18 1.7 µ 50 x 2.1 mm; Eluent A: 1 l Water + 1.0 mL (25%ig Ammonia)/L,

Eluent B: 1 l Acetonitrile; Gradient: 0.0 min 92% A → 0.1 min 92% A → 1.8 min 5% A → 3.5 min 5% A; Oven: 50°C; Flow: 0.45 mL/min; UV-Detection: 210 nm.

LC/MS-Method 5: Instrument: Agilent MS Quad 6150; HPLC: Agilent 1290; Column: Waters Acquity UPLC HSS T3 1.8 µm 50 x 2.1 mm; Eluent A: 1 l Water + 0.25 ml 99 %ige Formic acid, Eluent B: 1 l Acetonitrile + 0.25 ml 99 %ige Formic acid; Gradient: 0.0 min 90 % A → 0.3 min 90 % A → 1.7 min 5 % A → 3.0 min 5 % A Oven: 50°C; Flow: 1,20 ml/min; UV-Detection: 205 – 305 nm.

LC/MS-Method 6: Instrument MS: ThermoFisherScientific LTQ-Orbitrap-XL; Instrument HPLC: Agilent 1200SL; Column: Agilent, POROSHELL 120, 3 x 150 mm, SB – C18 2.7 µm; Eluent A: 1 l water + 0.1 % TFA; Eluent B: 1 l acetonitrile + 0.1 % TFA; Gradient: 0.0 min 2 % B → 0.3 min 2 % B → 5.0 min 95 % B → 10.0 min 95 % B; Oven: 40°C; flow: 0.75 ml/min; UV-detection: 210 nm.

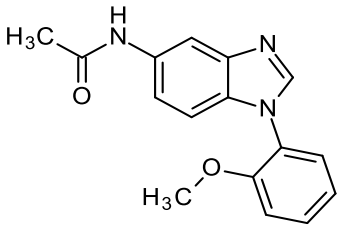
Single Mass Analysis (HR-MS): Instrument: Waters Time of Flight System (ToF), Electrospray Ionization (ESI).

Ion chromatography: Quantitative analysis of cations and anions with external standards. Instrument: Thermo Scientific ICS 5000+; Kapillar IC Column: IonPac AS11-HC and IonPac CS16; Eluent: Gradient Eluent $[H]^+$ $[OH]^-$; Detector: conductivity detection.

Synthetic procedures

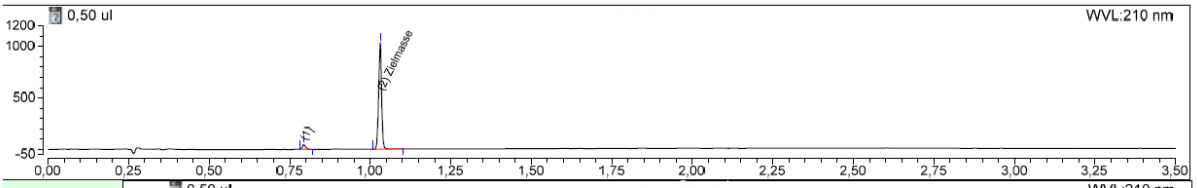
Preparation of (1):

N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]acetamide:



1-(2-methoxyphenyl)-1H-benzimidazol-5-amine (200 mg, 0.84 mmol) was dissolved in 5 ml of pyridine. Acetyl chloride (0.07 ml, 0.92 mmol) was added to the mixture and the mixture was stirred for 2 h at room temperature. The crude product was purified by preparative HPLC (Instrument: Waters Prep LC/MS System, Column: XBridge C18 5 µm 100x30 mm. Eluent A: water, eluent B: acetonitrile, eluent C: 2 % ammonia in water, eluent D: acetonitrile/water (80 Vol.%/20 Vol%) flow: 80 ml/min, room temperature, detection: 200-400 nm, At-Column injection. Gradient: eluent A 0 to 2 min 63 ml, eluent B 0 to 2 min 7 ml, eluent A 2 to 10 min from 63 ml to 39 ml and eluent B from 7 ml to 31 ml, 10 to 12 min 0 ml eluent A and 70 ml eluent B. Eluent C and eluent D constant flow each 5 ml/min over the whole runtime). Product containing samples were united and the mixture was lyophilized. 175 mg (96 % purity, 71 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.03 min; MS (ESIpos): m/z = 282 $[M+H]^+$

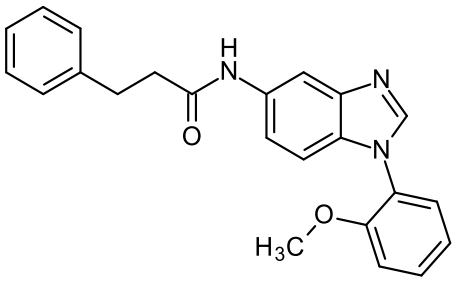


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,79	4,4	n.a.		
2	Y	1,03	95,6	100,0	282,1237	

^{1}H-NMR (600 MHz, DMSO-d_6) δ [ppm] = 9.94 (s, 1H), 8.28 (s, 1H), 8.06 (m, 1H), 7.54-7.48 (m, 2H), 7.38-7.32 (m, 2H), 7.17-7.14 (m, 2H), 3.78 (s, 3H), 2.54 (s, 3H).

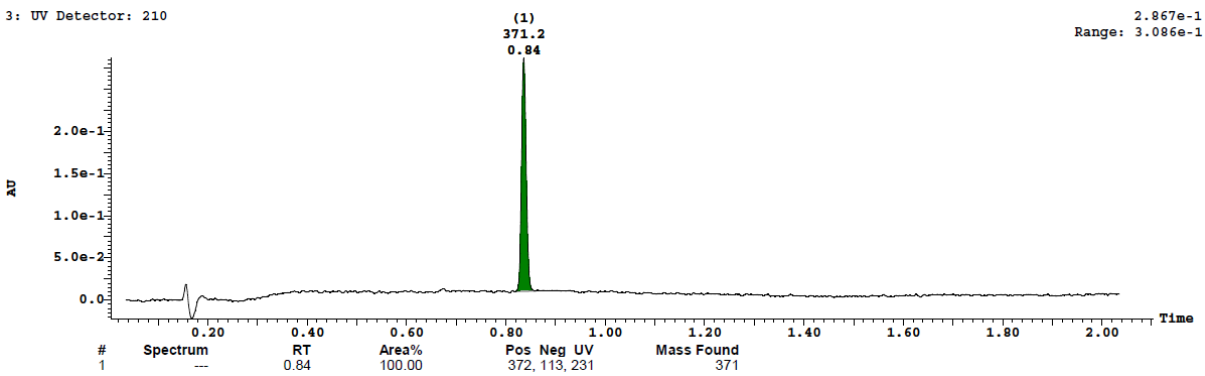
Preparation of (2):

N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]-3-phenylpropanamide:



3-Phenylpropanoic acid (77 µl, 0.47 mmol) was dissolved in 3.75 ml of DMF. HATU (153 mg, 0.40 mmol) and DIPEA (19 µl, 1.07 mmol) were added to the mixture and the mixture was stirred for 30 min at room temperature. Then 1-(2-methoxyphenyl)-1H-benzimidazol-5-amine (80 mg, 0.33 mmol) was added to the mixture and the reaction mixture was stirred at room temperature for 1 h. The crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 72 mg (100 % purity, 59 % yield) of the title compound were obtained.

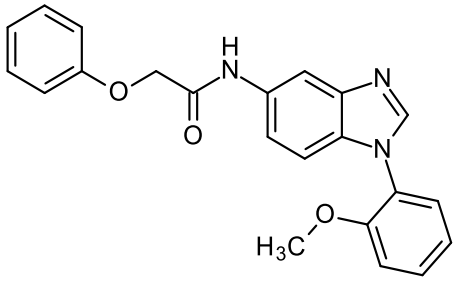
LC-MS (Method 1): Rt = 0.84 min; MS (ESIpos): m/z = 372 $[M+H]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.97 (s, 1H), 8.48 (s, 1H), 8.11 (s, 1H), 7.56-7.51 (m, 2H), 7.35-7.15 (m, 9H), 3.79 (s, 3H), 2.94 (t, J = 7.95 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H).

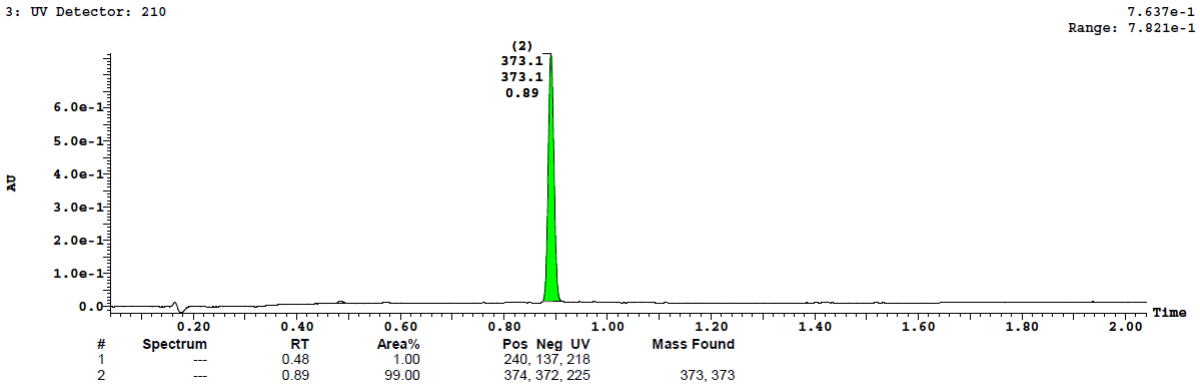
Preparation of (3):

N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]-2-phenoxyacetamide:



1-(2-methoxyphenyl)-1H-benzimidazol-5-amine (130 mg, 0.54 mmol) was dissolved in 9.63 ml of acetonitrile. Triethylamine (24 µl, 1.68 mmol) was added to the mixture and the reaction was cooled to 0 °C. At this temperature phenoxyacetyl chloride (77 µl, 0.55 mmol) was added to the mixture and the reaction mixture was stirred at room temperature overnight. The crude product was acidified with 1N HCl and purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 120 mg (99 % purity, 58 % yield) of the title compound were obtained.

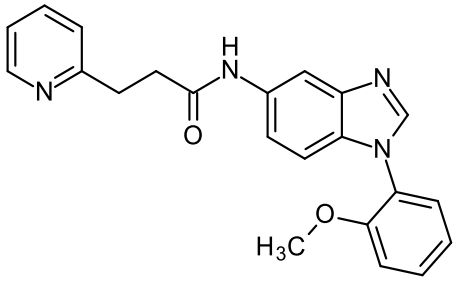
LC-MS (Method 1): Rt = 0.89 min; MS (ESIpos): m/z = 374 $[M+H]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.09 (s, 1H), 8.31 (s, 1H), 8.11 (s, 1H), 7.55-7.46 (m, 3H), 7.35-7.31 (m, 3H), 7.20-7.14 (m, 2H), 7.05-6.97 (m, 3H), 4.71 (s, 2H), 3.79 (s, 3H).

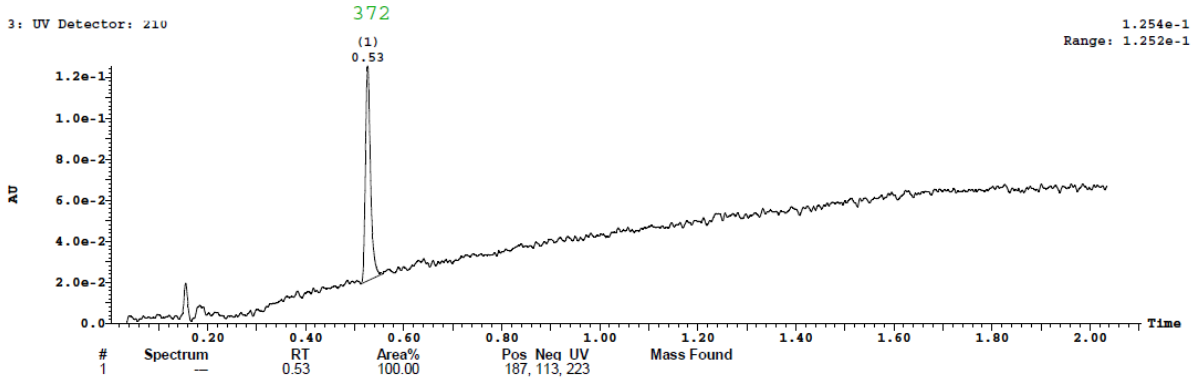
Preparation of (4):

N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]-3-(pyridin-2-yl)propanamide:



3-(pyridin-2-yl)propanoic acid (88 mg, 0.59 mmol) was dissolved in 3.75 ml of DMF. HATU (190 mg, 0.50 mmol) and DIPEA (36 µl, 2.09 mmol) were added to the mixture and the mixture was stirred for 30 min at room temperature. Then 1-(2-methoxyphenyl)-1H-benzimidazol-5-amine (100 mg, 0.42 mmol) was added to the mixture and the reaction mixture was stirred at room temperature for 1 h. The mixture was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated. The crude product was purified by preparative HPLC (Instrument: Waters Prep LC/MS System, Column: XBridge C18 5 µm 100x30 mm. Eluent A: water, eluent B: acetonitrile, eluent C: 2 % ammonia in water, eluent D: acetonitrile/water (80 Vol.%/20 Vol%) flow: 80 ml/min, room temperature, detection: 200-400 nm, At-Column injection. Gradient: eluent A 0 to 2 min 63 ml, eluent B 0 to 2 min 7 ml, eluent A 2 to 10 min from 63 ml to 39 ml and eluent B from 7 ml to 31 ml, 10 to 12 min 0 ml eluent A and 70 ml eluent B. Eluent C and eluent D constant flow each 5 ml/min over the whole runtime). Product containing samples were united and the solvents were lyophilized. 24 mg (100 % purity, 15 % yield) of the title compound were obtained.

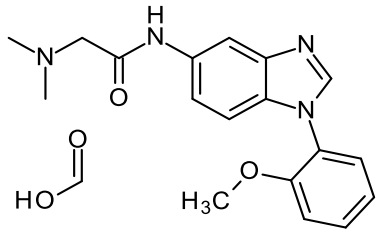
LC-MS (Method 1): Rt = 0.53 min; MS (ESIpos): m/z = 372 $[M]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.97 (s, 1H), 8.49 (m, 1H), 8.27 (s, 1H), 8.06 (d, J = 1.71 Hz, 1H), 7.70 (td, J = 7.67 Hz, 1H), 7.54-7.47 (m, 2H), 7.39-7.30 (m, 3H), 7.22-7.13 (m, 3H), 3.78 (s, 3H), 3.08 (m, 2H), 2.78 (t, J = 7.64 Hz, 2H).

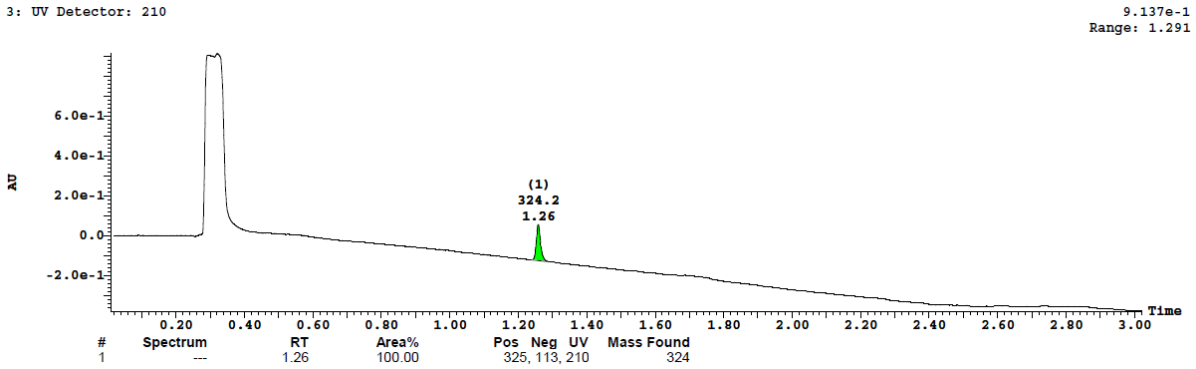
Preparation of (5):

formic acid-N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]-N^2,N^2-dimethylglycinamide:



2-chloro-N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]acetamide (6) (100 mg, 0.32 mmol) was dissolved in 3 ml of DMF, potassium iodide (158 mg, 0.95 mmol) was added and the mixture was stirred at 50°C for 30 min. Then N-methylmethanamine hydrochloride (77 mg, 0.95 mmol) and DIPEA (170 µl, 0.95 mmol) were added to the mixture and the reaction mixture was stirred 20 h at 50°C. The crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 20 mg (100 % purity, 17 % yield) of the title compound were obtained.

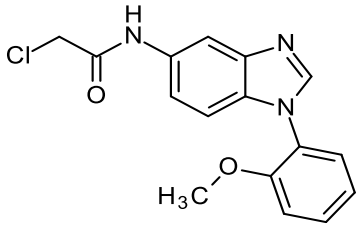
LC-MS (Method 4): Rt = 1.26 min; MS (ESIpos): m/z = 324 $[M+H-HCOO]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.90 (br. s, 1H), 8.30 (s, 1H), 8.12 (m, 2H), 7.55-7.43 (m, 3H), 7.33 (d, J = 8.19 Hz, 1H), 7.18-7.16 (m, 2H), 3.78 (s, 3H), 2.43 (s, 6H).

Preparation of (6):

2-chloro-N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]acetamide:



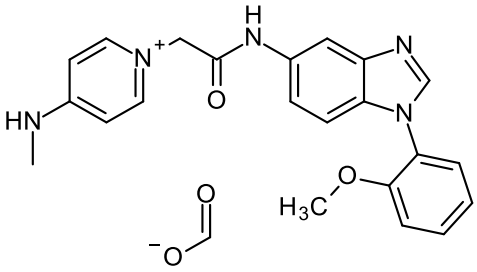
1-(2-methoxyphenyl)-1H-benzimidazol-5-amine (500 mg, 1.84 mmol) was dissolved in 13.2 ml of dichloromethane. Triethylamine (280 µl, 2.02 mmol) was added to the mixture and the reaction was cooled to 0 °C. At this temperature chloroacetyl chloride (150 µl, 1.93 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and acidified with 1 N HCl. The mixture was then extracted with ethyl acetate. Conc. $NaHCO_3$- solution was added to the aqueous layer and the mixture was extracted with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered and evaporated. 512 mg (95 % purity, 84 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.25 min; MS (ESIpos): m/z = 316 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.32 (s, 1H), 8.32 (s, 1H), 8.07 (d, J = 1.71 Hz, 1H), 7.55-7.47 (m, 2H), 7.41-7.32 (m, 2H), 7.21-7.14 (m, 2H), 4.28 (s, 2H), 3.79 (s, 3H).

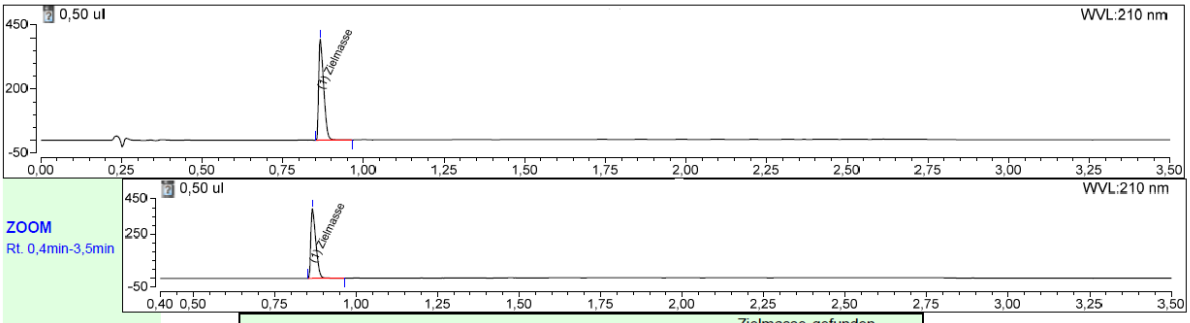
Preparation of (8):

1-(2-{[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate:



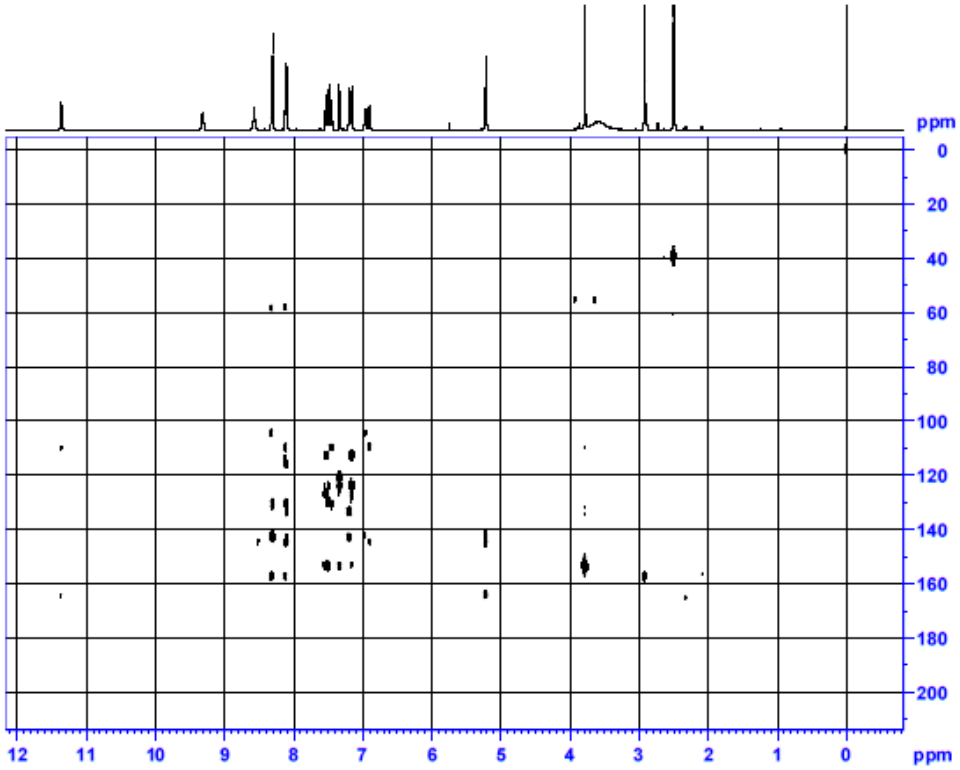
2-chloro-N-[1-(2-methoxyphenyl)-1H-benzimidazol-5-yl]acetamide (6) (250 mg, 89 % purity, 0.70 mmol) was dissolved in 7.5 ml of DMF, potassium iodide (117 mg, 0.70 mmol) was added and the mixture was stirred at 50°C for 30 min. Then N-methylpyridin-4-amine (228 mg, 2.11 mmol) and DIPEA (368 µl, 2.11 mmol) were added and the mixture was stirred for 30 min at 50°C. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; Gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 50 ml/min; 0.1% formic acid). Product containing samples were united and the solvents were evaporated. 101 mg (100 % purity, 33 % yield) of the title compound were obtained.

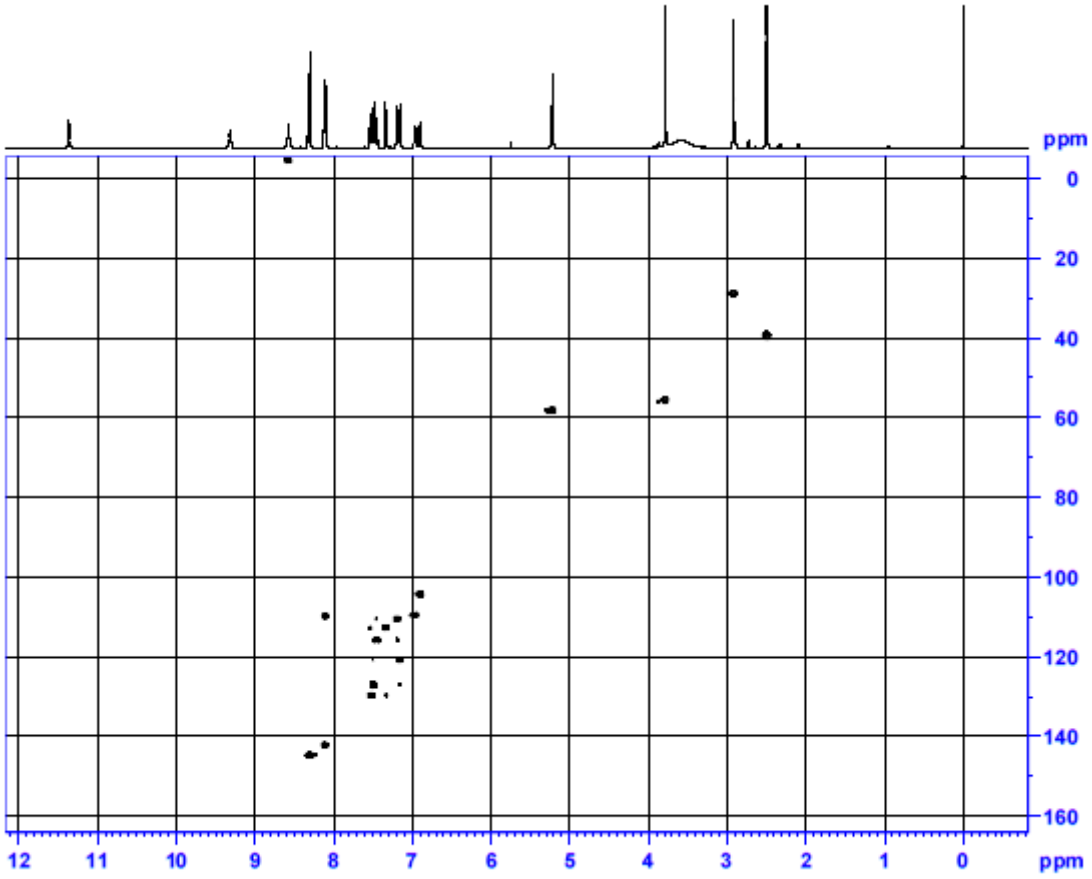
LC-MS (Method 2): Rt = 0.8 min; MS (ESIpos): m/z = 388 $[M-HCOO]^+$

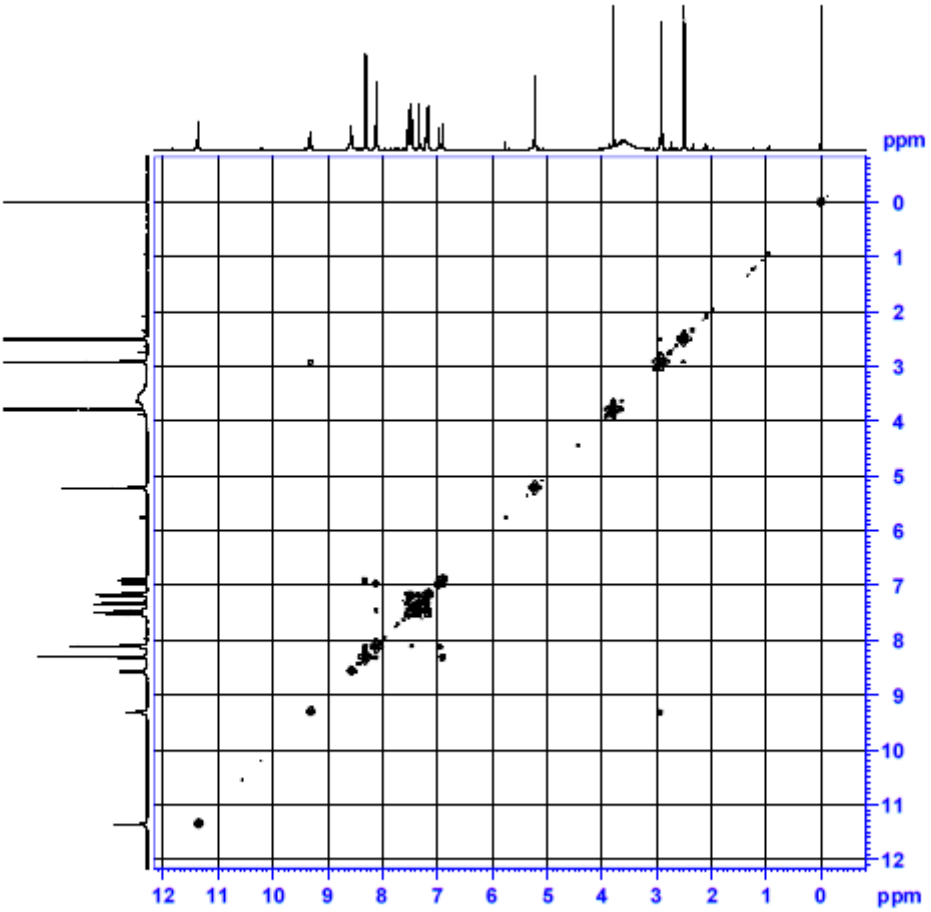


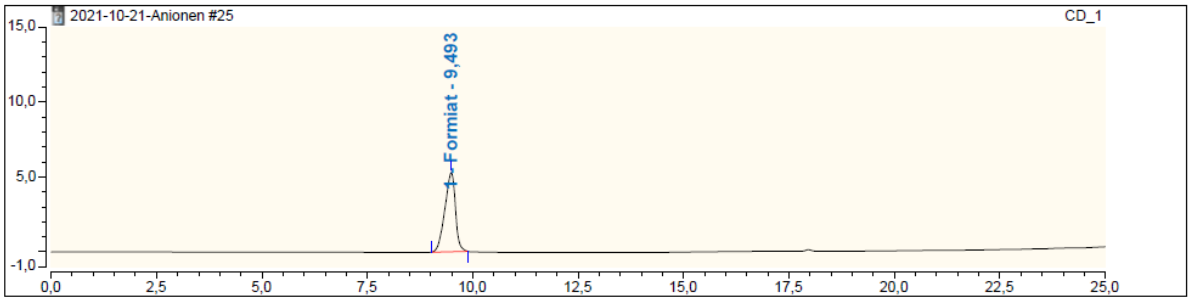
			Zielmasse gefunden			
#	Spectrum	RT	Area%	Area %	[M]+	[M-H]-
		min	210nm	UVMax	Pos	Neg
1	Y	0,87	100,0	100,0	388,1768	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.30 (s, 1H), 9.27 (s, 1H), 8.58 (s, 1H), 8.31 (m, 1H), 8.12-8.09 (m, 2H), 7.54-7.45 (m, 3H), 7.34 (d, J = 8,31 Hz, 1H), 7.20-7.16 (m, 2H), 6.95 (d, J = 7.34 Hz, 2H), 5.22 (s, 2H), 3.79 (s, 3H), 2.92 (s, 3H).




ppm
0
20
40
60
80
100
120
140
160
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



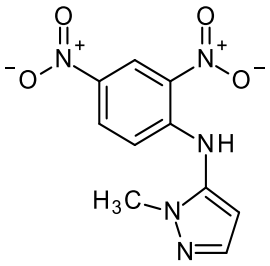


No.	Name	Time min	Massenanteil in (Gew. %) Formiat
23	Leer	n.a.	n.a.
25		9,49	9,3
26		9,48	9,7
	Average:	9,49	9,5

Ion chromatography: w(formate) = 9.5 weight % = 1.1 eq. (formate).

Preparation of (9) and respective starting materials:

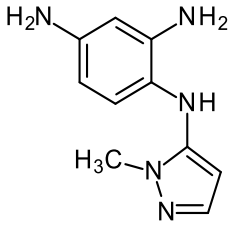
N-(2,4-dinitrophenyl)-1-methyl-1H-pyrazol-5-amine:



1-methyl-1H-pyrazol-5-amine (574 mg, 5.91 mmol) was dissolved in 30 ml of 1,4-dioxane. Potassium tert-butoxide (904 mg, 8.06 mmol) was added and the mixture was stirred at room temperature for 10 min. Then 1-fluoro-2,4-dinitrobenzene (1 g, 5.37 mmol) was added and the mixture was stirred overnight at 80 °C. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP KP-Sil 50 g Ultra; eluent: Cy / EE: 12 % EE ->100 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 446 mg (100 % purity, 32 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.80 min; MS (ESIpos): m/z = 264 $[M+H]^+$

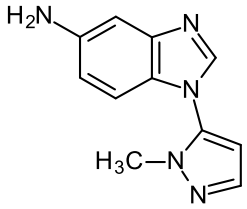
N-(1-methyl-1H-pyrazol-5-yl)benzene-1,2,4-triamine:



N-(2,4-dinitrophenyl)-1-methyl-1H-pyrazol-5-amine (446 mg, 1.69 mmol) was dissolved in 7 ml of acetic acid. Tin (II) chloride dihydrate (2.68 g, 11.86 mmol) dissolved in 7 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. The crude product (243 mg) was used in the next step without further purification.

LC-MS (Method 4): Rt = 1.09 min; MS (ESIpos): m/z = 203 $[M]^+$

1-(1-methyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-amine:

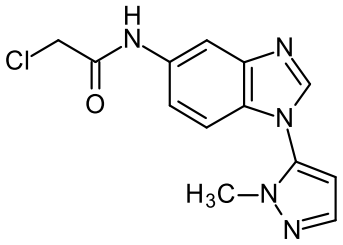


Crude N-(1-methyl-1H-pyrazol-5-yl)benzene-1,2,4-triamine (243 mg) was dissolved in 5.35 ml of 4 N hydrochloric acid. Formic acid (130 µl, 3.35 mmol) was added and the mixture was stirred at 80 °C overnight. Further formic acid (130 µl, 3.35 mmol) was added and the mixture was stirred at 80 °C for 7 days. The reaction mixture was adjusted to pH 9 with 4 N sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium

sulfate, filtered and evaporated. The crude product (95 mg, 45 % purity) was used in the next step without further purification.

LC-MS (Method 4): Rt = 0.83 min; MS (ESIpos): m/z = 214 $[M+H]^+$

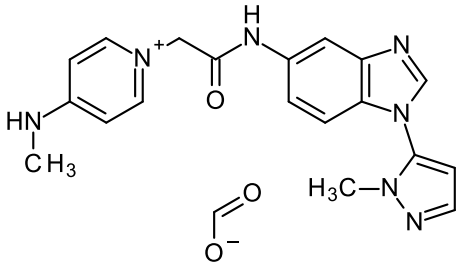
2-chloro-N-[1-(1-methyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]acetamide:



1-(1-methyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-amine (95 mg, 45 % purity, 0.20 mmol) was dissolved in 2 ml of dichloromethane. Triethylamine (30 µl, 0.22 mmol) was added and the reaction was cooled to 0 °C. At this temperature chloroacetyl chloride (20 µl, 0.21 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with water and acidified with 1 N HCl. The mixture was then extracted with ethyl acetate. Conc. $NaHCO_3$- solution was added to the aqueous layer and the mixture was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 94 mg (76 % purity) of the title compound were obtained.

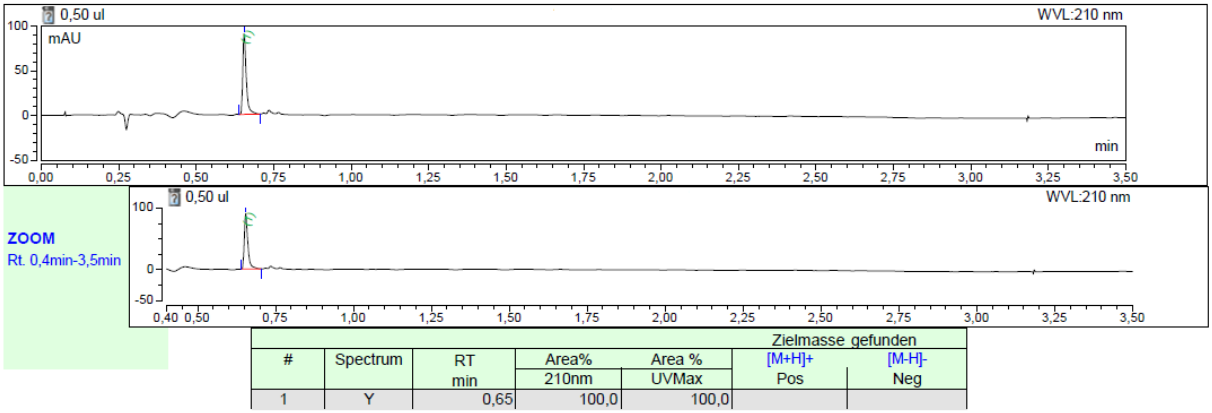
LC-MS (Method 2): Rt = 0.96 min; MS (ESIpos): m/z = 290 $[M+H]^+$

4-(methylamino)-1-(2-{[1-(1-methyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]amino}-2-oxoethyl)pyridinium formate (9):

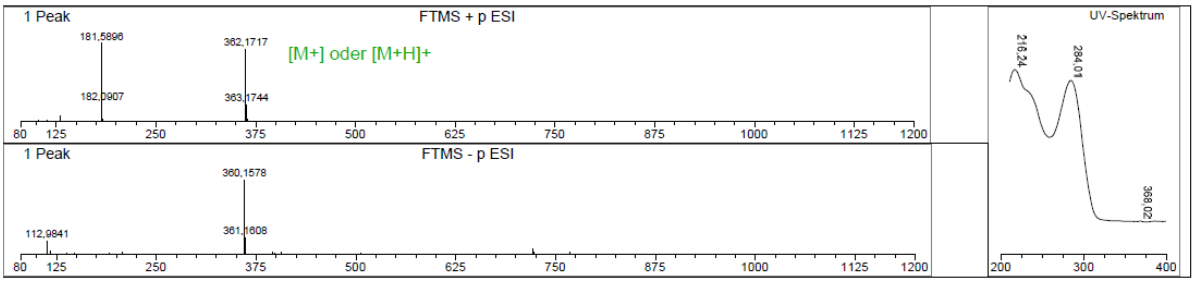


2-chloro-N-[1-(1-methyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]acetamide (94 mg, 76 % purity, 0.32 mmol) was dissolved in 3.3 ml of DMF, potassium iodide (54 mg, 0.32 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (105 mg, 0.97 mmol) and DIPEA (170 µl, 0.97 mmol) were added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP KP-NH 28 g; eluent: DCM / MeOH: 5 % MeOH ->40 % MeOH; flow: 75 ml/min). Product containing samples were united and the solvents were evaporated. Further purification by preparative HPLC was needed (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; Gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 50 ml/min; 0.1% formic acid). Product containing samples were united and the solvents were evaporated. 7 mg (100 % purity, 5.3 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.65 min; MS (ESIpos): m/z = 362 $[M\text{-}HCOO]^+$



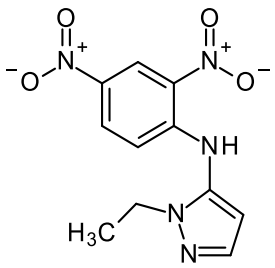
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,65	100,0	100,0		



^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 11.52 (s, 1H), 9.29 (s, 1H), 8.53-7.99 (m, 5H), 7.60 (m, 2H), 7.30 (s, 1H), 6.96 (m, 2H), 6.61 (s, 1H), 5.24 (s, 2H), 3.66 (s, 3H), 2.92 (s, 3H).

<u>Preparation of (10) and respective starting materials:</u>

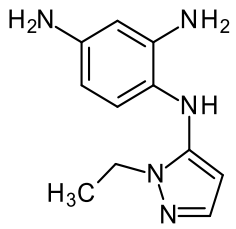
N-(2,4-dinitrophenyl)-1-ethyl-1H-pyrazol-5-amine:



1-ethyl-1H-pyrazol-5-amine (1.31 g, 11.82 mmol) was dissolved in 60 ml of 1,4-dioxane. Potassium tert-butoxide (1.81 g, 16.12 mmol) was added and the mixture was stirred at room temperature for 10 min. Then 1-fluoro-2,4-dinitrobenzene (2 g, 10.75 mmol) was added and the mixture was stirred overnight at 80 °C. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: Cy/EE 4:1). Product containing samples were united and the solvents were evaporated. 1.09 g (55 % purity, 20 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.61 min; MS (ESIpos): m/z = 278 $[M+H]^+$

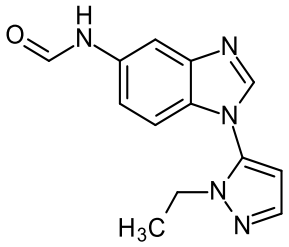
N-(1-ethyl-1H-pyrazol-5-yl)benzene-1,2,4-triamine:



N-(2,4-dinitrophenyl)-1-ethyl-1H-pyrazol-5-amine (1.09 g, 55 % purity, 2.16 mmol) was dissolved in 10 ml of acetic acid. Tin (II) chloride dihydrate (1.7 g, 7.56 mmol) dissolved in 10 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice water, basified with a 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. 603 mg (57 % purity, 73 % yield) of the title compound were obtained.

LC-MS (Method 4): Rt = 0.85 min; MS (ESIpos): m/z = 217 $[M+H]^+$

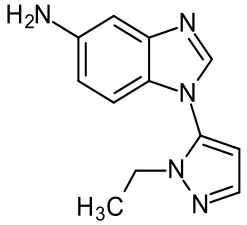
N-[1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]formamide:



N-(1-ethyl-1H-pyrazol-5-yl)benzene-1,2,4-triamine (510 mg, 57 % purity, 1.34 mmol) was dissolved in 20 ml of trimethoxymethane. The mixture was stirred at 100 °C overnight. The solvent was evaporated and the crude product (542 mg, 62 % purity) was used in the next step without further purification.

LC-MS (Method 4): Rt = 0.86 min; MS (ESIpos): m/z = 256 $[M+H]^+$

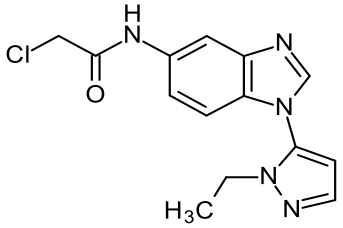
1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-amine:



N-[1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]formamide (542 mg, 62 % purity) was dissolved in 20 ml of methanol / conc. hydrochloric acid 1:1 and the mixture was stirred at 100 °C overnight. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. 293 mg (72 % purity, 44 % yield) of the title compound were obtained.

LC-MS (Method 4): Rt = 0.27 min; MS (ESIpos): m/z = 228 $[M+H]^+$

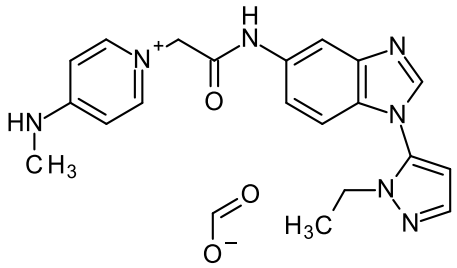
2-chloro-N-[1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]acetamide:



1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-amine (193 mg, 72 % purity, 0.61 mmol) was dissolved in 5 ml of dichloromethane. Triethylamine (90 µl, 0.67 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (50 µl, 0.64 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated and the residue was stirred with acetonitrile. The suspension was poured into water. The precipitate was filtered off and discarded. The filtrate was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 63 mg (100 % purity, 34 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.06 min; MS (ESIpos): m/z = 304 $[M+H]^+$

1-(2-{[1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (10):



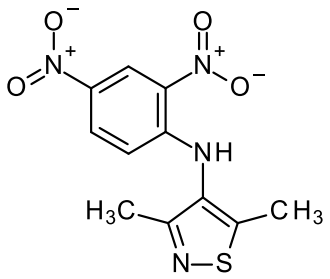
2-chloro-N-[1-(1-ethyl-1H-pyrazol-5-yl)-1H-benzimidazol-5-yl]acetamide (63 mg, 0.21 mmol) was dissolved in 3 ml of DMF, potassium iodide (34 mg, 0.21 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (67 mg, 0.62 mmol) and DIPEA (110 µl, 0.62 mmol) were added and the mixture was stirred at 50 °C overnight. The crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 22 mg (92 % purity, 23 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.45 min; MS (ESIpos): m/z = 376 $[M-HCOO]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.81 (s, 1H), 8.84 (s, 1H), 8.48 (s, 1H), 8.41 (s, 2H), 8.31 (d, J = 1.1 Hz, 1H), 8.13-8.09 (m, 2H), 7.71 (d, J = 2.08 Hz, 1H), 7.49 (d, J = 8.31 Hz, 1H), 7.25 (d, J = 8.93 Hz, 1H), 6.95-6.85 (m, 2H), 6.61 (d, J = 1.96 Hz, 1H), 5.17 (s, 1H), 3.48 (q, J = 7.17 Hz, 2H), 2.94 (d, J = 5.01 Hz, 3H), 1.20 (t, J = 7.27 Hz, 3H).

Preparation of (11) and respective starting materials:

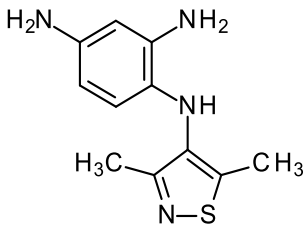
N-(2,4-dinitrophenyl)-3,5-dimethyl-1,2-thiazol-4-amine:



3,5-dimethyl-1,2-thiazol-4-amine (1.0 g, 7.80 mmol) and 1-fluoro-2,4-dinitrobenzene (1.6 g, 8.58 mmol) were dissolved in 20 ml of NMP. DIPEA (2.85 ml, 16.38 mmol) was added and the mixture was stirred at 110 °C overnight. The reaction mixture was diluted with water and stirred for 30 min. The precipitate was filtered off, washed with water and dried in vacuo. 751 mg (70 % purity, 23 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.83 min; MS (ESIpos): m/z = 295 $[M+H]^+$

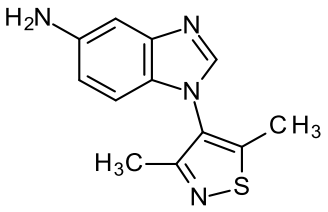
N-(3,5-dimethyl-1,2-thiazol-4-yl)benzene-1,2,4-triamine:



N-(2,4-dinitrophenyl)-3,5-dimethyl-1,2-thiazol-4-amine (751 mg, 70 % purity, 1.77 mmol) was dissolved in 4.89 ml of acetic acid. Tin (II) chloride dihydrate (2.00 g, 8.87 mmol) dissolved in 4.89 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature overnight. Additional tin (II) chloride dihydrate (1.00 g, 4.43 mmol) dissolved in 3 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred 2 h at room temperature. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. 550 mg (81 % purity, 107 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.53 min; MS (ESIpos): m/z = 235 $[M+H]^+$

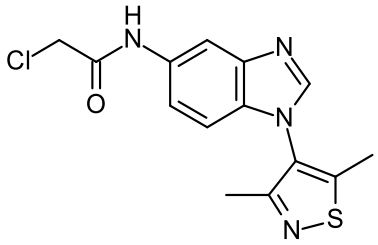
1-(3,5-dimethyl-1,2-thiazol-4-yl)-1H-benzimidazol-5-amine:



N-(3,5-dimethyl-1,2-thiazol-4-yl)benzene-1,2,4-triamine (550 mg, 81 % purity, 1.91 mmol) was dissolved in 18.42 ml of 4 N hydrochloric acid. Formic acid (200 µl, 5.34 mmol) was added and the mixture was stirred at 80 °C overnight. The reaction mixture was adjusted to pH 9 with 4 N sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 450 mg (91 % purity, 88 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.64 min; MS (ESIpos): m/z = 245 $[M+H]^+$

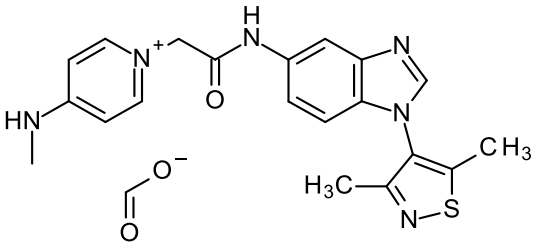
2-chloro-N-[1-(3,5-dimethyl-1,2-thiazol-4-yl)-1H-benzimidazol-5-yl]acetamide:



1-(3,5-dimethyl-1,2-thiazol-4-yl)-1H-benzimidazol-5-amine (450 mg, 91% purity) was dissolved in 15 ml of dichloromethane. Triethylamine (280 µl, 2.03 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (150 µl, 1.93 mmol) was added and the reaction was stirred for 30 min at room temperature. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$ solution and brine, dried over sodium sulfate, filtered and evaporated. The crude product (549 mg, 75 % purity) was used in the next step without further purification.

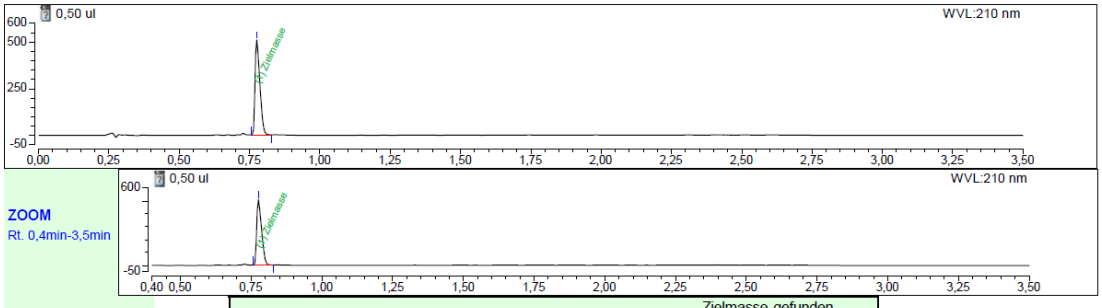
LC-MS (Method 2): Rt = 1.17 min; MS (ESIpos): m/z = 321 $[M+H]^+$

1-(2-{[1-(3,5-dimethyl-1,2-thiazol-4-yl)-1H-benzimidazol-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (11):



2-chloro-N-[1-(3,5-dimethyl-1,2-thiazol-4-yl)-1H-benzimidazol-5-yl]acetamide (150 mg, 75 % purity) was dissolved in 3 ml of DMF, potassium iodide (78 mg, 0.47 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (152 mg, 1.40 mmol) and DIPEA (240 µl, 1.40 mmol) were added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; Gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 50 ml/min; 0.1% formic acid). Product containing samples were united and the solvents were evaporated. 116 mg (100 % purity, 84 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.78 min; MS (ESIpos): m/z = 393 $[M-HCOO]^+$

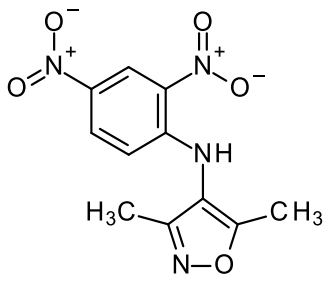


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,78	100,0	100,0	393,1493	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.01-10.94 (m, 1H), 8.93 (br. s, 1H), 8.40 (s, 2H), 8.31-8,29 (m, 1H), 8.11-8.90 (m, 2H), 7.44 (d, J = 8.8 Hz, 1H), 7.21 (d, J = 8.68 Hz, 1H), 6.93 (d, J = 7.58 Hz, 1H), 5.19 (s, 3H), 2.92 (d, J = 4.77 Hz, 3H), 2.30 (s, 3H), 2.13 (s, 3H).

Preparation of (12) and respective starting materials:

N-(2,4-dinitrophenyl)-3,5-dimethyl-1,2-oxazol-4-amine:

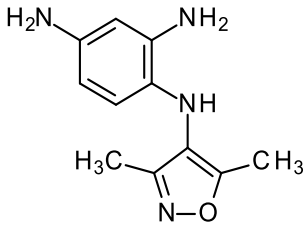


3,5-dimethyl-1,2-oxazol-4-amine (4.9 g, 43.70 mmol) and 1-fluoro-2,4-dinitrobenzene (7.4 g, 39.73 mmol) were dissolved in 100 ml of NMP. DIPEA (14.53 ml, 83.42 mmol) was added and the mixture was stirred at 110 °C overnight. The reaction mixture was diluted with water and stirred for 30 min. The precipitate was filtered off, washed with water and dried in vacuo. 9.53 g (100 % purity, 86 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.93 min; MS (ESIneg): m/z = 277 $[M-H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9,62 (s, 1H), 8.90 (d, J = 2.69 Hz, 1H), 8.23 (dd, J = 9.48 Hz, 1H), 6.85 (d, J = 9.41 Hz, 1H), 2.28 (s, 3H), 2.08 (s, 3H).

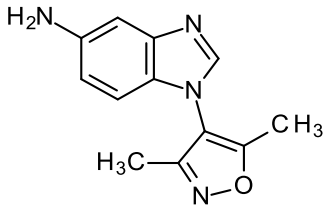
N-(3,5-dimethyl-1,2-oxazol-4-yl)benzene-1,2,4-triamine:



N-(2,4-dinitrophenyl)-3,5-dimethyl-1,2-oxazol-4-amine (39.63 g, 141.47 mmol) was dissolved in 440 ml of acetic acid. Tin (II) chloride dihydrate (223.45 g, 990.27 mmol) dissolved in 440 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. Activated charcoal and magnesium sulfate were added to the combined organic layers. The mixture was filtered, and the solvent was evaporated. 26.08 g (93 % purity, 78 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.21 min; MS (ESIpos): m/z = 218 $[M]^+$

1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-amine:

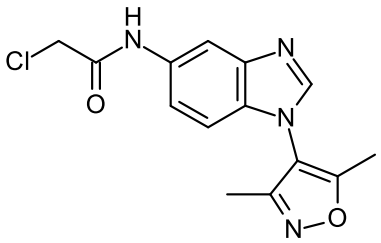


N-(3,5-dimethyl-1,2-oxazol-4-yl)benzene-1,2,4-triamine (26.08 g, 93 % purity, 110.98 mmol) was dissolved in 750 ml of 4 N hydrochloric acid. Formic acid (11.72 ml, 310.75 mmol) was added and the mixture was stirred at 80 °C overnight. The reaction mixture was basified with sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: EE/MeOH 10:1). Product containing samples were united and the solvents were evaporated. 10.92 g (100 % purity, 43 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.56 min; MS (ESIpos): m/z = 229 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.12 (s, 1H), 6.97 (d, J = 8.56 Hz, 1H), 6.87 (d, J = 1.83 Hz, 1H), 6.65 (dd, J = 8.56 Hz, 1H), 4.88 (s, 2H), 2.31 (s, 3H), 2.08 (s, 3H).

2-chloro-N-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]acetamide:

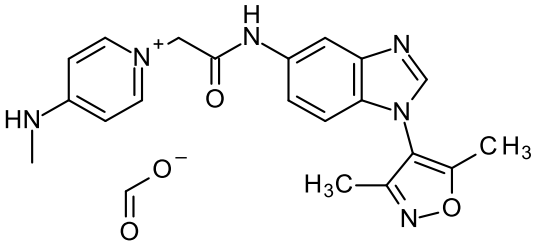


1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-amine (1.89 g, 99 % purity, 8.25 mmol) was dissolved in 60 ml of dichloromethane. Triethylamine (1.26 ml, 9.07 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (690 µl, 8.66 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$- solution and brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: EE). Product containing samples were united and the solvents were evaporated. 1.71 g (100 % purity, 68 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.12 min; MS (ESIpos): m/z = 305 $[M+H]^+$

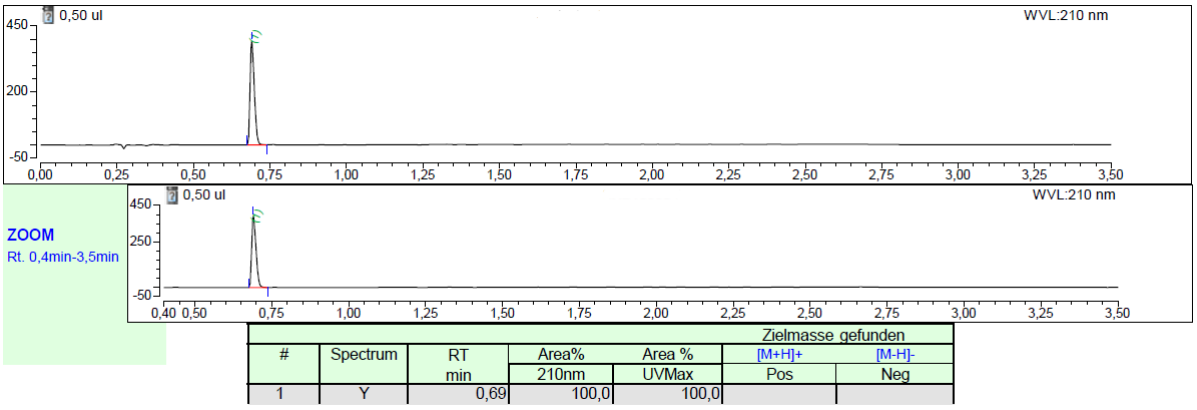
^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 10.36 (s, 1H), 8.40 (s, 1H), 8.10 (d, J = 1.73 Hz, 1H), 7.44 (dd, J = 8.67 Hz, 1H), 7.34 (d, J = 8.67 Hz, 1H), 4.28 (s, 2H), 2.53 (s, 3H), 2.10 (s, 3H).

1-(2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (12):

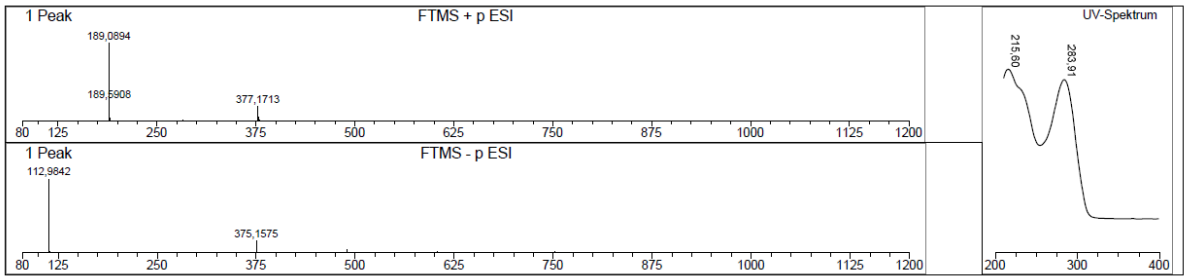


2-chloro-N-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]acetamide (1 g, 3.28 mmol) was dissolved in 30 ml of DMF, potassium iodide (545 mg, 3.28 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (1.06 g, 9.85 mmol) and DIPEA (1.72 ml, 9.85 mmol) were added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 1.1 g (100 % purity, 79 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.69 min; MS (ESIpos): m/z = 377 [M-HCOO]$^+$



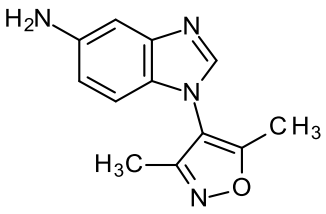
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,69	100,0	100,0		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.08-11.04 (m, 1H), 9.01 (br. s, 1H), 8.39 (d, J = 6.48 Hz, 2H), 8.30 (d, J = 6.6 Hz, 1H), 8.11-8.09 (m, 2H), 7.48 (d, J = 9.29 Hz, 1H), 7.32 (d, J = 8.68 Hz, 1H), 6.91 (d, J = 7.46 Hz, 2H), 5.20 (s, 2H), 2.93 (d, J = 4.89 Hz, 3H), 2.83 (s, 3H), 2.10 (s, 3H).

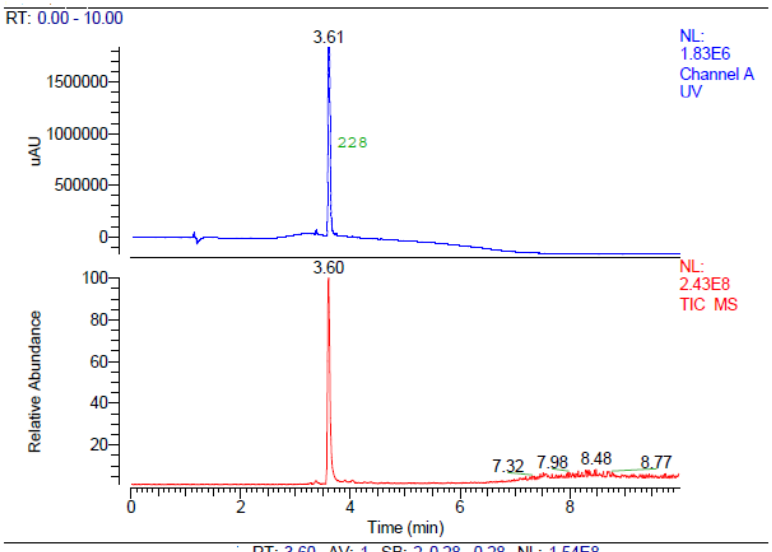
Preparation of (13) and respective starting materials:

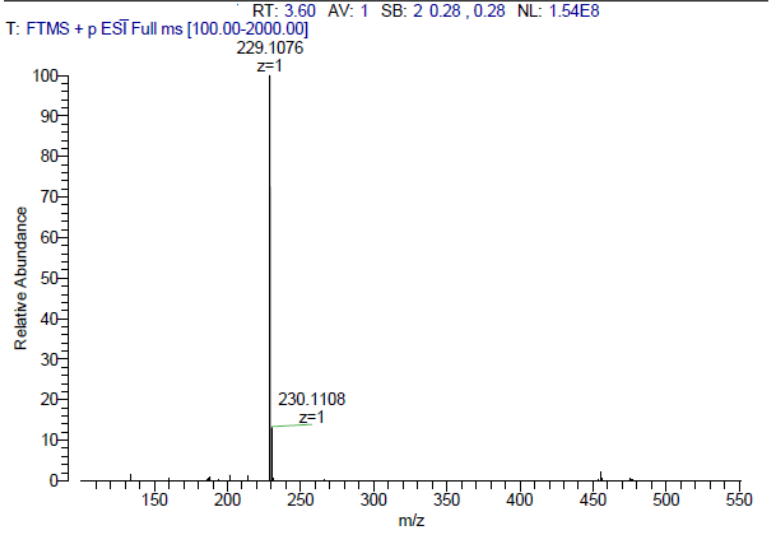
1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-amine (13):



N-(3,5-dimethyl-1,2-oxazol-4-yl)benzene-1,2,4-triamine (46.1 g, 91 % purity, 191.1 mmol) was dissolved in 1.2 l of 4 N hydrochloric acid. Formic acid (20.2 ml, 535.1 mmol) was added and the mixture was stirred at 80 °C overnight. The reaction mixture was basified with sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: EE/MeOH 10:1). Product containing samples were united and the solvents were evaporated. 10.2 g (100 % purity, 23 % yield) of the title compound were obtained.

105 mg of the title compound were further purified by preparative HPLC (Column: Daicel Chiralpak AS-H 5 µm; 250x20 mm; eluent: 50 % n-heptane & 50 % i-propanol). Product containing samples were united and the solvents were evaporated. 76 mg (100 % purity; 73 % yield) of the title compound were obtained.

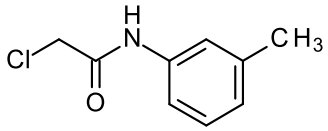




^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.12 (m, 1H), 6.98 (m, 1H), 6.87 (m, 1H), 6.63 (m, 1H), 4.87 (br. s, 2H), 2.31 (s. 3H), 2.08 (s, 3H).

Preparation of (14) and respective starting materials:

2-chloro-N-(3-methylphenyl)acetamide:

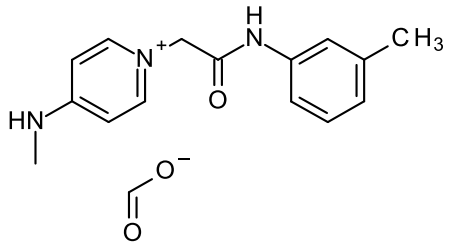


m-Toluidine (250 mg, 2.33 mmol) was dissolved in 17.75 ml of dichloromethane. Triethylamine (0.36 ml, 2.57 mmol) was added, and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (0.20 ml, 2.45 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$-solution and brine, dried over sodium sulfate, filtered, and evaporated. 470 mg (100 % purity, 110 % yield) of the title compound were obtained.

LC-MS (Method 5): Rt = 1.01 min; MS (ESIpos): m/z = 184 $[M+H]^+$

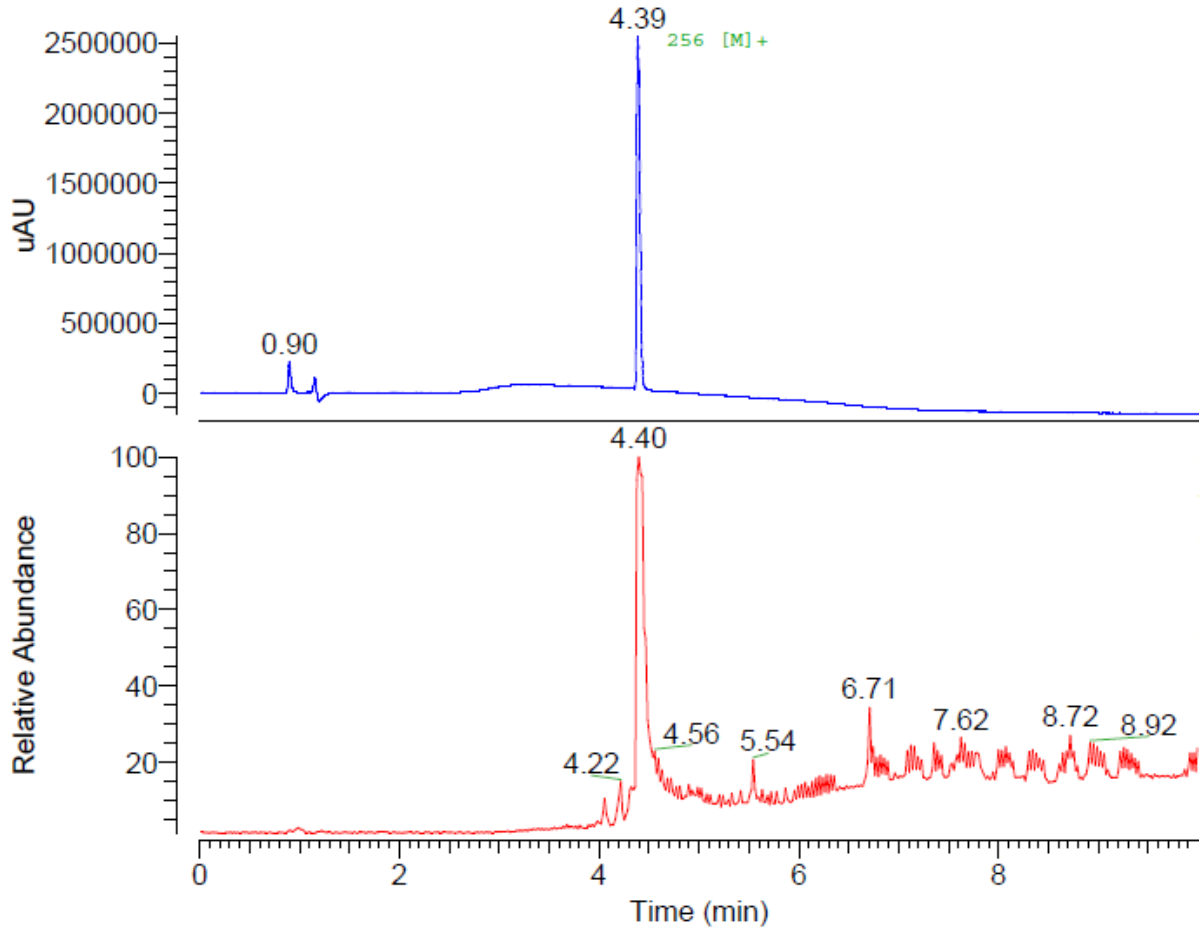
^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.09 (br s, 1H), 7.41 (s, 1H), 7.36 (d, J = 8.19 Hz, 1H), 7.21 (t, 1H), 6.9 (d, J = 7.58 Hz, 1H), 4.28 (s. 2H), 2.28 (s, 3H).

4-(methylamino)-1-[2-(3-methylanilino)-2-oxoethyl]pyridinium formate (14):



2-Chloro-N-(3-methylphenyl)acetamide (470 mg, 2.56 mmol) was dissolved in 22.46 ml of DMF, potassium iodide (545 mg, 3.28 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (408 mg, 2.46 mmol) and DIPEA (1.28 ml, 7.37 mmol) were added, and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 705 mg (100 % purity, 91 % yield) of the title compound were obtained.

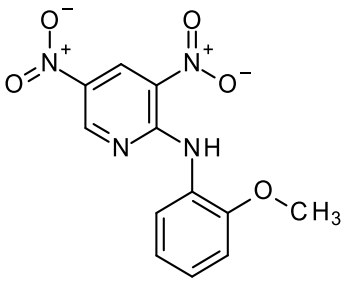
LC-MS (Method 6): Rt = 4.40 min; MS (ESIpos): m/z = 256 $[M\text{-}HCOO]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 10.82-10.61 (m, 1H), 8.98-8.85 (m, 1H), 8.55 (s, 1H), 8.26 (d, J = 7.09 Hz, 1H), 8.05 (d, J = 7.09 Hz, 1H), 7.43 (s, 1H), 7.36 (d, J = 8.07 Hz, 1H), 7.21 (t, J = 7.82 Hz, 1H), 6.92-6.90 (m, 3H), 5.13 (s, 2H), 2.92 (d, J = 3.91 Hz, 3H), 2.27 (s, 3H).

<u>Preparation of (17) and respective starting materials:</u>

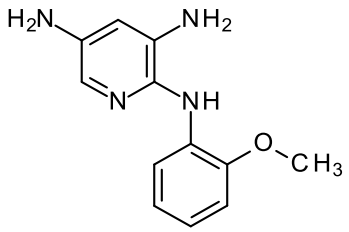
N-(2-methoxyphenyl)-3,5-dinitropyridin-2-amine:



2-Methoxyaniline (610 µl, 5.40 mmol) and 2-chloro-3,5-dinitropyridine (1.0 g, 4.91 mmol) were dissolved in 10 ml of acetonitrile and stirred at room temperature overnight. The precipitate was filtered off, washed with water, and dried in vacuo. 886 mg (100 % purity, 62 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 2.06 min; MS (ESIpos): m/z = 291 $[M+H]^+$

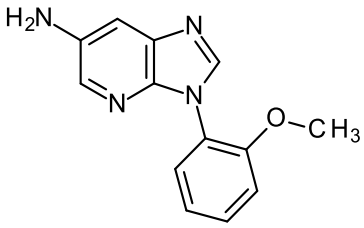
N-(2-methoxyphenyl)pyridine-2,3,5-triamine:



N-(2-Methoxyphenyl)-3,5-dinitropyridin-2-amine (886 mg, 3.05 mmol) was dissolved in 10 ml of ethyl acetate, Palladium (10% on activated carbon, 325 mg, 0.31 mmol) was added, and the mixture was stirred under a hydrogen atmosphere overnight. The catalyst was filtered off through Celite, the filtrate was concentrated, and the residue was dried in vacuo. 515 mg (100 % purity, 73 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.51 min; MS (ESIpos): m/z = 231 $[M+H]^+$

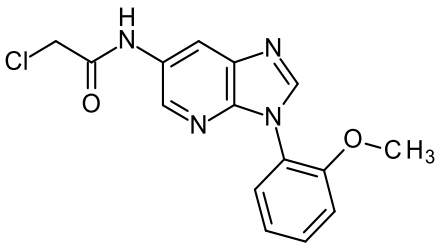
3-(2-methoxyphenyl)-3H-imidazo[4,5-b]pyridin-6-amine:



N-(2-Methoxyphenyl)pyridine-2,3,5-triamine (1.68 g, 7.31 mmol) was dissolved in 35 ml of 4 N hydrochloric acid. Formic acid (0.77 ml, 20.47 mmol) was added, and the mixture was stirred at 80 °C overnight. The reaction mixture was basified with sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. 1.48 g (88 % purity, 74 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.81 min; MS (ESIpos): m/z = 241 $[M+H]^+$

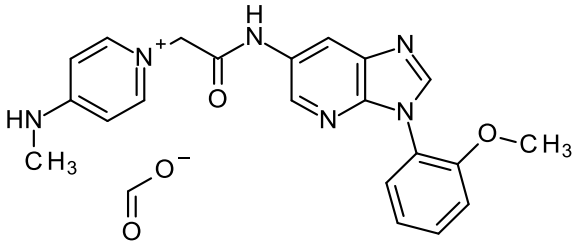
2-chloro-N-[3-(2-methoxyphenyl)-3H-imidazo[4,5-b]pyridin-6-yl]acetamide:



3-(2-Methoxyphenyl)-3H-imidazo[4,5-b]pyridin-6-amine (456 mg, 80 % purity, 1.52 mmol) was dissolved in 15 ml of dichloromethane. Triethylamine (290 µl, 2.09 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (160 µl, 1.99 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$-solution and brine, dried over sodium sulfate, filtered and evaporated. 585 mg (78 % purity, 94 % yield) of the title compound were obtained.

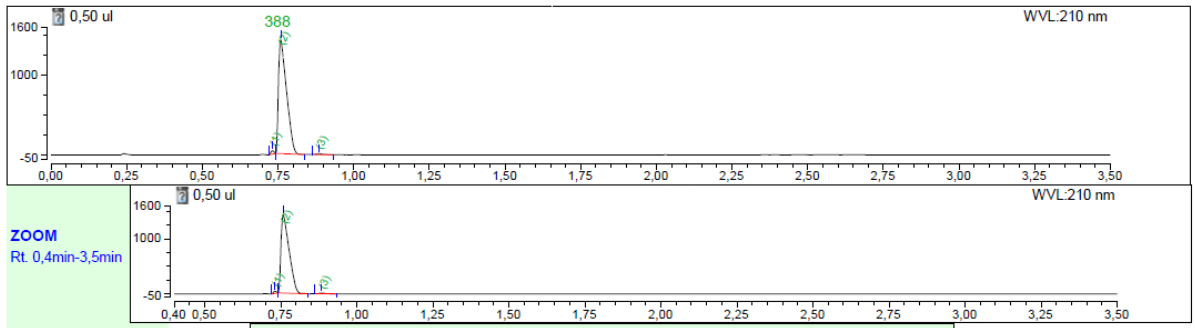
LC-MS (Method 2): Rt = 1.19 min; MS (ESIpos): m/z = 317 $[M+H]^+$

1-(2-{[3-(2-methoxyphenyl)-3H-imidazo[4,5-b]pyridin-6-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (17):

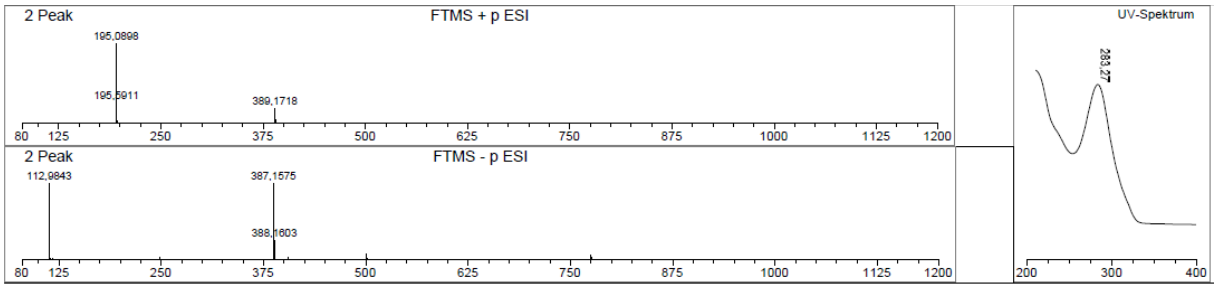


2-Chloro-N-[3-(2-methoxyphenyl)-3H-imidazo[4,5-b]pyridin-6-yl]acetamide (150 mg, 78 % purity, 0.37 mmol) was dissolved in 9.5 ml of DMF, potassium iodide (79 mg, 0.47 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (154 mg, 1.42 mmol) was added, and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 70 mg (99 % purity, 43 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.76 min; MS (ESIpos): m/z = 388 $[M\text{-}HCOO]^+$



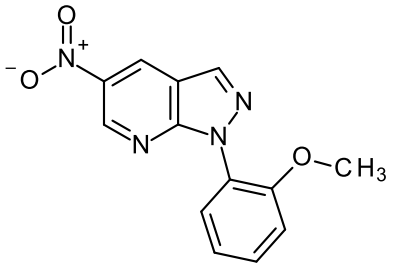
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,73	1,1	1,2		
2	Y	0,76	98,2	98,8		
3	Y	0,88	0,7	n.a.		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.37 (m, 1H), 8.99 (m, 1H), 8.56 (s, 1H), 8.50 (s, 1H), 8.44 (br s, 2H), 8.31 (d, J = 6.48 Hz, 1H), 8.11 (d, J = 6.24 Hz, 1H), 7.58-7.51 (m, 2H), 7.32 (d, J = 8.19 Hz, 1H), 7.16 (t, J = 7.58 Hz, 1H), 6.92 (d, J = 7.46 Hz, 2H), 5.20 (s, 2H), 3.77 (s, 3H), 2.93 (d, J = 4.77 Hz, 3H).

Preparation of (18) and respective starting materials:

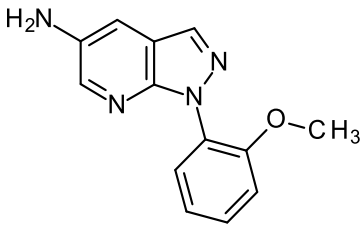
1-(2-methoxyphenyl)-5-nitro-1H-pyrazolo[3,4-b]pyridine:



5-Nitro-1H-pyrazolo[3,4-b]pyridine (100 mg, 0.61 mmol) was treated with (2-methoxyphenyl)boronic acid (185 mg, 1.22 mmol), copper (II) acetate monohydrate (36.5 mg, 0.18 mmol) and pyridine (0.10 ml, 1.22 mmol) in 1.22 ml of DMF and stirred overnight at 30 °C. The reaction mixture was acidified with formic acid, diluted with water and extracted 3 times with ethyl acetate. The combined organic layers are dried over sodium sulfate, filtered, and evaporated. The crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 20.3 mg (100 % purity, 12 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.66 min; MS (ESIpos): m/z = 271 $[M+H]^+$

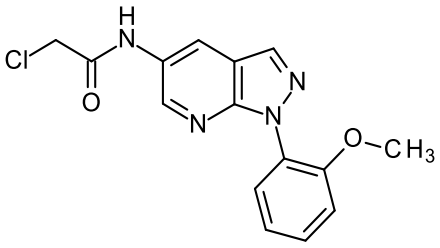
1-(2-methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-5-amine:



1-(2-Methoxyphenyl)-5-nitro-1H-pyrazolo[3,4-b]pyridine (20.3 mg; 0.08 mmol) was dissolved in 2.51 ml of acetic acid. Tin(II)chloride dihydrate (84.75 mg, 0.38 mmol) dissolved in 2.51 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred 2 h at room temperature. The reaction mixture was poured onto ice water, basified with a 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 9 mg (100 % purity, 50 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.51 min; MS (ESIpos): m/z = 241 $[M+H]^+$

2-chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-5-yl]acetamide:

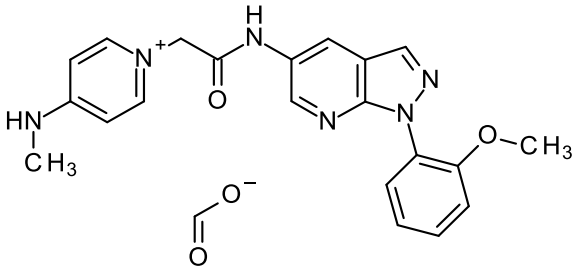


1-(2-Methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-5-amine (9 mg, 0.04 mmol) was dissolved in 0.27 ml of dichloromethane. Triethylamine (5.74 µl, 0.04 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (3.13 µl, 0.04 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with

dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$ solution and brine, dried over sodium sulfate, filtered and evaporated. 10.7 mg (100 % purity, 90 % yield) of the title compound were obtained.

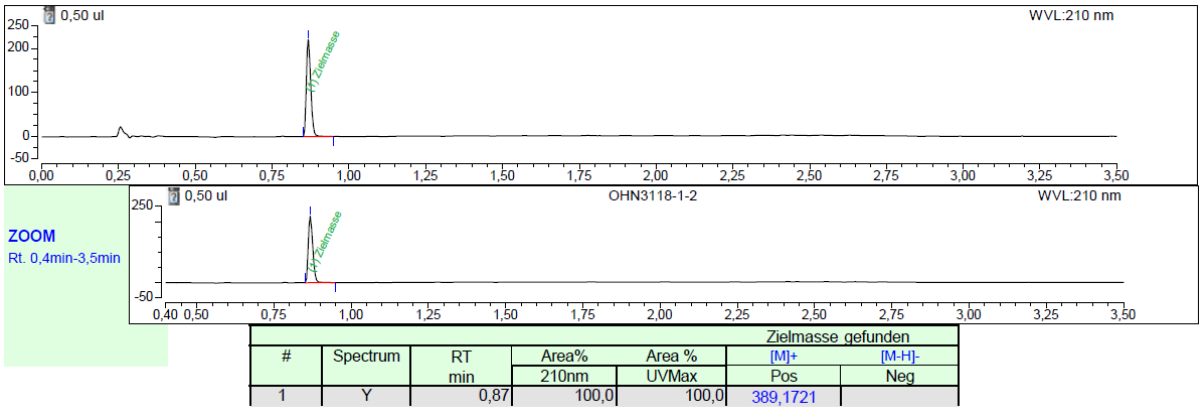
LC-MS (Method 2): Rt = 1.29 min; MS (ESIpos): m/z = 317 $[M+H]^+$

1-(2-{[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (18):



2-Chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-5-yl]acetamide (10.7 mg, 0.03 mmol) was dissolved in 0.42 ml of DMF, potassium iodide (5.6 mg, 0.03 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (10.96 mg, 0.1 mmol) and DIPEA (20 µl, 0.1 mmol) were added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 8.1 mg (100 % purity, 55 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.87 min; MS (ESIpos): m/z = 389 $[M-HCOO]^+$

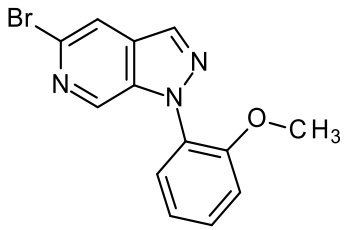


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,87	100,0	100,0	389,1721	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.11 (m, 1H), 8.86 (m, 1H), 8.60-8.55 (m, 3H), 8.32 (d, J = 6.97 Hz, 2H), 8.08 (d, J = 6.97 Hz, 1H), 7.54 (m, 1H), 7.41 (dd, J = 7.82 Hz, 1H), 7.26 (d, J = 8,31 Hz, 1H), 7.13 (t, J = 7.52 Hz, 1H), 6.94-6.89 (m, 2H), 5.21 (s, 2H), 3.70 (s, 3H), 2.93 (s, 3H).

Preparation of (19) and respective starting materials:

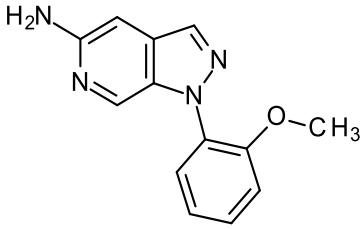
5-bromo-1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridine:



5-Bromo-1H-pyrazolo[3,4-c]pyridine (100 mg, 0.51 mmol) was treated with (2-methoxyphenyl)boronic acid (153.5 mg, 1.01 mmol), copper (II) acetate monohydrate (30.2 mg, 0.15 mmol) and pyridine (82 µl, 1.01 mmol) in 1 ml of DMF and stirred overnight at 30 °C. The reaction mixture was acidified with formic acid, diluted with water and extracted 3 times with ethyl acetate. The combined organic layers are dried over sodium sulfate, filtered, and evaporated. The crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 77.9 mg (98 % purity, 50 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.93 min; MS (ESIpos): m/z = 303 $[M+H]^+$

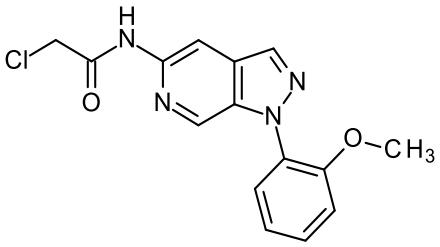
1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-5-amine:



5-Bromo-1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridine (77.9 mg, 98 % purity, 0.51 mmol), tris (dibenzylidene acetone) dipalladium (17.2 mg, 0.02 mmol), 1,1'-binaphthalene-2,2'-diylbis (diphenylphosphane) (23.4 mg, 0.04 mmol) and 1,1-diphenylmethanimine (72.8 mg, 0.40 mmol) were placed in 1.6 ml of DME under an argon atmosphere. Then cesium carbonate (204.5 mg, 0.63 mmol) was added and the mixture was stirred at 115 °C overnight. The reaction mixture was filtered through celite, rinsed with ethyl acetate and the filtrate was concentrated. The residue was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 30.3 mg (9 % purity, 5 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.52 min; MS (ESIpos): m/z = 241 $[M+H]^+$

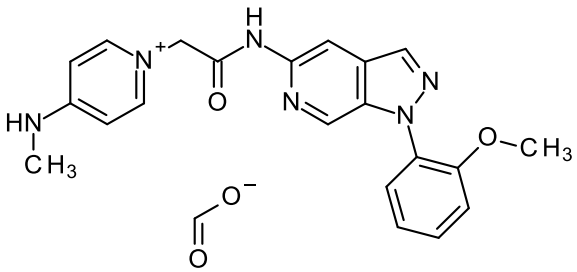
2-chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-5-yl]acetamide:



1-(2-Methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-5-amine (30.3 mg, 9 % purity, 0.01 mmol) was dissolved in 1 ml of dichloromethane. Triethylamine (19.3 µl, 0.14 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (10.6 µl, 0.13 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 3 mg (100 % purity, 95 % yield) of the title compound were obtained.

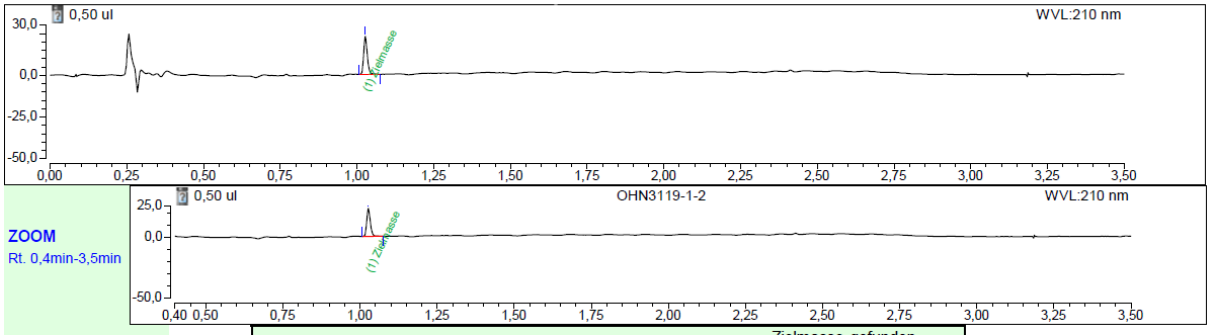
LC-MS (Method 2): Rt = 1.55 min; MS (ESIpos): m/z = 317 $[M+H]^+$

1-(2-{[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (19):



2-Chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[3,4-c]pyridin-5-yl]acetamide (3 mg, 0.01 mmol) was dissolved in 0.12 ml of DMF, potassium iodide (1.6 mg, 0.01 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (3.1 mg, 0.03 mmol) and DIPEA (10 µl, 0.03 mmol) were added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 0.6 mg (98 % purity, 14 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.03 min; MS (ESIpos): m/z = 389 $[M-HCOO]^+$

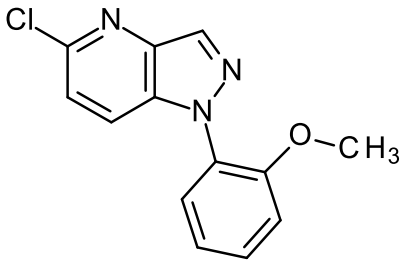


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	1,03	100,0	100,0	389,1721	

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 11.12 (s, 1H), 8.89 (m, 1H), 8.56 (m, 1H), 8.45-8.31 (m, 3H), 8.11 (d, J = 6.38 Hz, 1H), 7.59-7.49 (m, 2H), 7.33 (m, 1H), 7.17 (m, 1H), 6.91 (m, 2H), 5.20 (s, 2H), 3.82 (s, 3H), 2.93 (d, J = 4.57 Hz, 3H).

Preparation of (20) and respective starting materials:

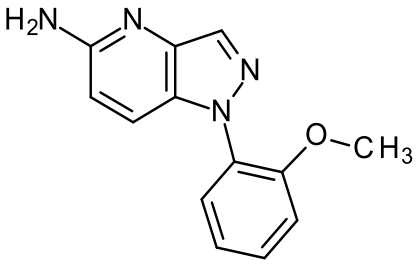
5-chloro-1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridine:



5-Chloro-1H-pyrazolo[4,3-b]pyridine (150 mg, 0.98 mmol) was treated with (2-methoxyphenyl)boronic acid (297 mg, 1.95 mmol), copper (II) acetate monohydrate (58.5 mg, 0.3 mmol) and pyridine (165 µl, 1.95 mmol) in 2 ml of DMF and stirred overnight at 30 °C. The reaction mixture was acidified with formic acid, diluted with water and extracted 3 times with ethyl acetate. The combined organic layers are dried over sodium sulfate, filtered, and evaporated. The crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 186 mg (78 % purity, 57 % yield) of the title compound were obtained.

LC-MS (Method 3): Rt = 2.68 min; MS (ESIpos): m/z = 260 $[M+H]^+$

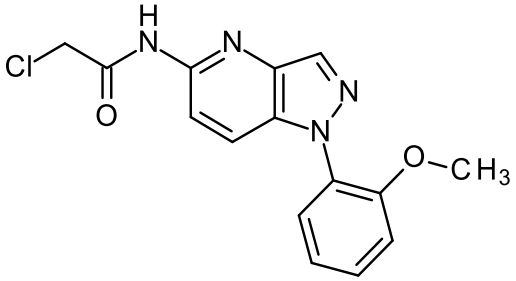
1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridin-5-amine:



5-Chloro-1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridine (120 mg, 78 % purity, 0.36 mmol), tris (dibenzylidene acetone) dipalladium (31.2 mg, 0.74 mmol), 1,1'-binaphthalene-2,2'-diylbis (diphenylphosphane) (43.2 mg, 0.07 mmol) and 1,1-diphenylmethanimine (134 mg, 0.74 mmol) were placed in 3 ml of DME under an argon atmosphere. Then cesium carbonate (376 mg, 1.15 mmol) was added, and the mixture was stirred at 115 °C overnight. The reaction mixture was filtered through celite, rinsed with ethyl acetate and the filtrate was concentrated. The residue was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 69 mg (29 % purity, 23 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.76 min; MS (ESIpos): m/z = 241 $[M+H]^+$

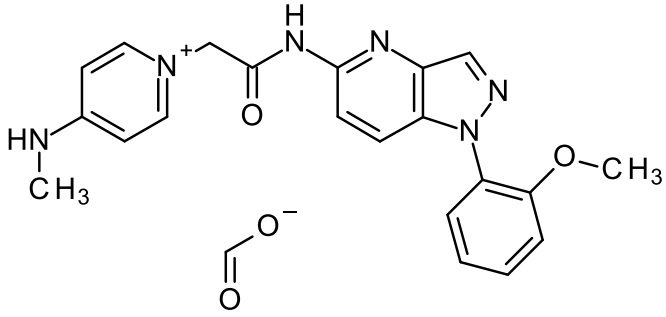
2-chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridin-5-yl]acetamide:



1-(2-Methoxyphenyl)-1H-pyrazolo[4,3-b]pyridin-5-amine (69 mg, 29 % purity, 0.09 mmol) was dissolved in 2 ml of dichloromethane. Triethylamine (44 µl, 0.32 mmol) was added and the reaction mixture was cooled to 0 °C. At this temperature chloroacetyl chloride (24 µl, 0.30 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with conc. $NaHCO_3$ solution and brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 23.8 mg (99 % purity, 83 % yield) of the title compound were obtained.

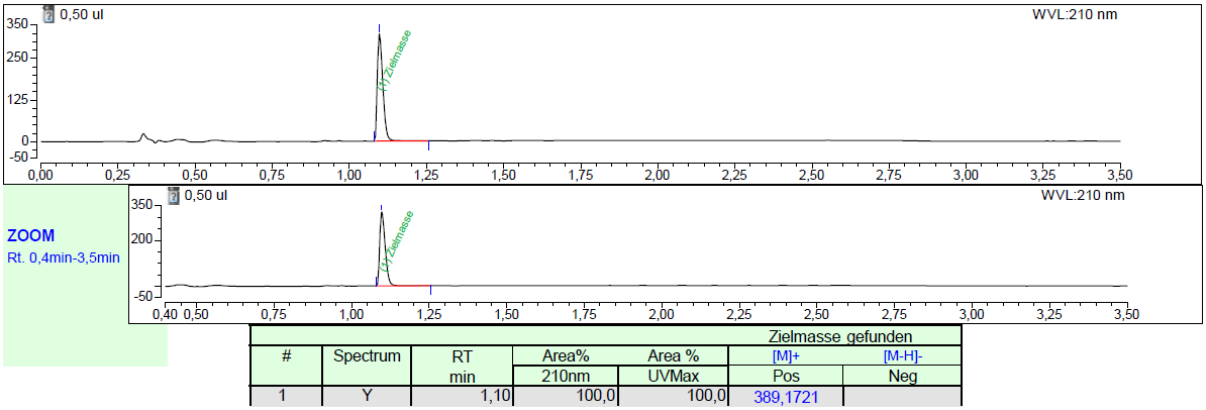
LC-MS (Method 2): Rt = 1.54 min; MS (ESIpos): m/z = 317 $[M+H]^+$

1-(2-{[1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridin-5-yl]amino}-2-oxoethyl)-4-(methylamino)pyridinium formate (20):



2-Chloro-N-[1-(2-methoxyphenyl)-1H-pyrazolo[4,3-b]pyridin-5-yl]acetamide (23 mg, 99 % purity, 0.07 mmol) was dissolved in 0.9 ml of DMF, potassium iodide (12 mg, 0.07 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (23.5 mg, 0.22 mmol) and DIPEA (40 µl, 0.22 mmol) were added and the mixture was stirred at 50 °C overnight. the reaction mixture was acidified with formic acid and the product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 17 mg (100 % purity, 54 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.10 min; MS (ESIpos): m/z = 389 [M-HCOO]$^+$

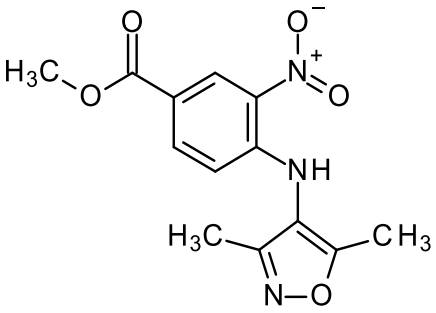


			Zielmasse gefunden			
#	Spectrum	RT min	Area% 210nm	Area % UVMax	[M]+ Pos	[M-H]- Neg
1	Y	1,10	100,0	100,0	389,1721	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.33 (br s, 1H), 9.03 (br s, 1H), 8.56 (s, 1H), 8.39 (s, 1H), 8.28 (d, J = 7.34 Hz, 1H), 8.10 (d, 7.34 Hz, 2H), 7.82 (d, J = 9.17 Hz, 1H), 7.57-7.48 (m, 2H), 7.33 (d, J = 7.95 Hz, 1H), 7.14 (t, J = 7.58 Hz, 1H), 6.92 (m, 2H), 5.23 (br s, 2H), 3.79 (s, 3H), 2.92 (s, 3H).

Preparation of (21) and respective starting materials:

methyl 4-[(3,5-dimethyl-1,2-oxazol-4-yl)amino]-3-nitrobenzoate:

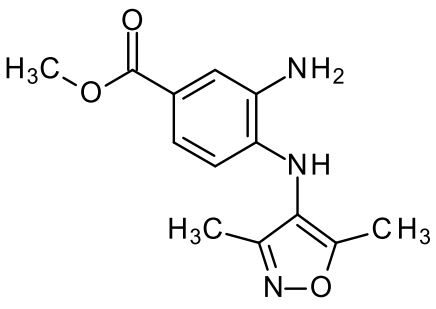


Methyl 4-fluoro-3-nitrobenzoate (7.5 g, 37.66 mmol) and 3,5-dimethyl-1,2-oxazol-4-amine (4.65 g, 41.43 mmol) were dissolved in 100 ml of NMP. DIPEA (13.78 ml, 79.09 mmol) was added, and the reaction mixture was stirred at 110 °C for 22 h. 900 ml of water were added and the mixture was stirred for 30 minutes. The precipitated solid was filtered off, washed with water and dried in vacuo. 9.29 g (100 % purity, 85 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.75 min; MS (ESIpos): m/z = 292 [M+H]$^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.38 (s, 1H), 8.66 (d, J = 2.08 Hz, 1H), 7.95 (dd, J = 8.93 Hz, 1H), 6.75 (d, J = 9.05 Hz, 1H), 3.84 (s, 3H), 2.27 (s, 3H), 2.06 (s, 3H).

methyl 3-amino-4-[(3,5-dimethyl-1,2-oxazol-4-yl)amino]benzoate:

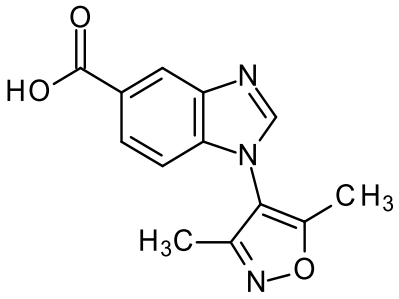


Methyl 4-[(3,5-dimethyl-1,2-oxazol-4-yl)amino]-3-nitrobenzoate (9.29 g, 31.90 mmol) was dissolved in 100 ml of acetic acid. Tin(II)chloride dihydrate (35.99 g, 159.48 mmol) dissolved in 100 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. Activated charcoal and magnesium sulfate were added to the combined organic layers. The mixture was filtered, and the solvent was evaporated. 7.01 g (100 % purity, 84 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 1.29 min; MS (ESIpos): m/z = 262 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 7.28 (d, J = 1.96 Hz, 1H), 7.12 (dd, J = 8.31 Hz, 1H), 6.72 (s, 1H), 6.13 (d, J = 8.31 Hz, 1H), 4.96 (s, 2H), 3.73 (s, 3H), 2.24 (s, 3H), 2.01 (s, 3H).

1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole-5-carboxylic acid:

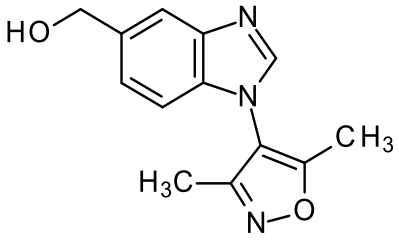


Methyl 3-amino-4-[(3,5-dimethyl-1,2-oxazol-4-yl)amino]benzoate (7.01 g, 26.83 mmol) was dissolved in 150 ml of 4 N hydrochloric acid. Formic acid (2.83 ml, 75.12 mmol) was added and the mixture was stirred at 80 °C for 71 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 5.37 g (91 % purity, 71 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.99 min; MS (ESIpos): m/z = 258 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 12.83 (br s, 1H), 8.57 (s, 1H), 8.35 (d, J = 1.1 Hz, 1H), 7.93 (dd, J = 8.56 Hz, 1H), 7.46 (d, J = 8.56 Hz, 1H), 2.34 (s, 3H), 2.11 (s, 3H).

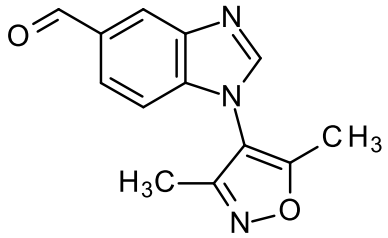
1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]methanol:



1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole-5-carboxylic acid (1.5 g, 91 % purity, 5.25 mmol) was dissolved in 45 ml of THF. Lithium aluminum hydride (376 mg, 9.9 mmol) was added in portions at room temperature. The mixture was stirred at room temperature for 4 days. The reaction mixture was quenched with water and 1 N sodium hydroxide solution was added until the precipitate was almost dissolved. The mixture was extracted with ethyl acetate, the combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. 786 mg (86 % purity, 48 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.89 min; MS (ESIpos): m/z = 244 $[M+H]^+$

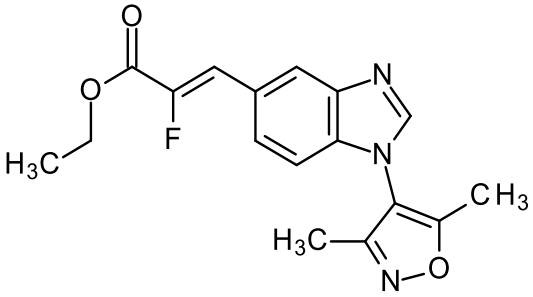
1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole-5-carbaldehyde:



1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]methanol (786 mg, 86 % purity, 2.78 mmol) was dissolved in 40 ml of chloroform. Manganese(IV)oxide (1.69 g, 19.39 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was filtered through celite, rinsed with dichloromethane and the filtrate was concentrated. The residue was dried in vacuo. 722 mg (83 % purity, 89 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.16 min; MS (ESIpos): m/z = 242 $[M+H]^+$

ethyl (2Z)-3-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-2-fluoroacrylate:

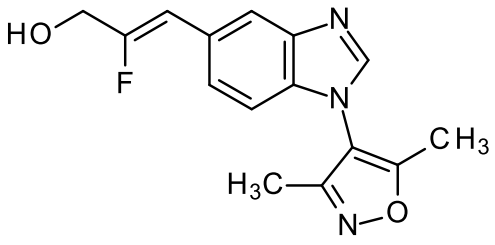


Under an argon atmosphere 1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole-5-carbaldehyde (646 mg, 83 % purity, 2.22 mmol) and ethyl (diethoxyphosphoryl)(fluoro)acetate (0.82 ml, 4.02 mmol) were dissolved in 7 ml of THF, and the mixture was cooled to -70 °C. At this temperature, N,N,N,N-tetramethylguanidine (0.55 ml, 4.42 mmol) was added dropwise. The reaction mixture was allowed to come to room temperature and stirred overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 25 g; eluent: Cy / EE: 12 % EE -> 100 % EE; flow: 75 ml/min). Product containing samples were united and the solvents were evaporated. 385 mg (100 % purity, 44 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.73 min; MS (ESIpos): m/z = 330 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.51 (s, 1H), 8.17 (s, 1H), 7.71 (dd, J = 8.62 Hz, 1H), 7.47 (d, J = 8.56 Hz, 1H), 7.31-7.22 (m, 1H), 4.31 (q, J = 7.09 Hz, 2H), 2.34 (s, 3H), 2.11 (s, 3H), 1.32 (t, J = 7.009 Hz, 3H).

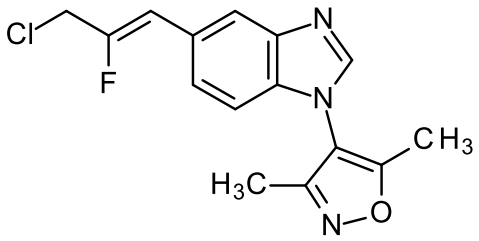
(2Z)-3-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-2-fluoroprop-2-en-1-ol:



Under an argon atmosphere calcium chloride (270 mg, 2.43 mmol) was dissolved in 7 ml of ethanol and the mixture was cooled to 0 °C. At this temperature a solution of ethyl (2Z)-3-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-2-fluoroacrylate (400 mg, 1.22 mmol) in 14 ml of THF and 7 ml of ethanol was added dropwise. The reaction mixture was stirred 10 min at 0 °C before sodium borohydride (184 mg, 4.86 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: Cy / EE: 16 % EE -> 100 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 196 mg (99 % purity, 56 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.17 min; MS (ESIpos): m/z = 288 $[M+H]^+$

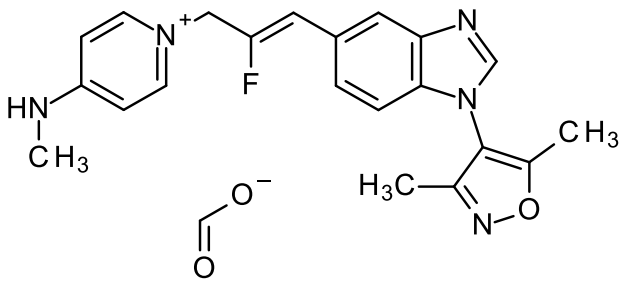
5-[(1Z)-3-chloro-2-fluoroprop-1-en-1-yl]-1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole:



(2Z)-3-[1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-2-fluoroprop-2-en-1-ol (196 mg, 99 % purity, 0.68 mmol) was dissolved in 3 ml of THF and the mixture was cooled to 0 °C. At this temperature thionyl chloride (0.12 ml, 1.71 mmol) was added dropwise. The reaction mixture was allowed to come to room temperature and stirred for 15 min. The reaction mixture was concentrated, and the residue was treated with diethyl ether. The precipitate was filtered off, washed with diethyl ether and dried in vacuo. 213 mg (62 % purity, 64 % yield) of the title compound were obtained.

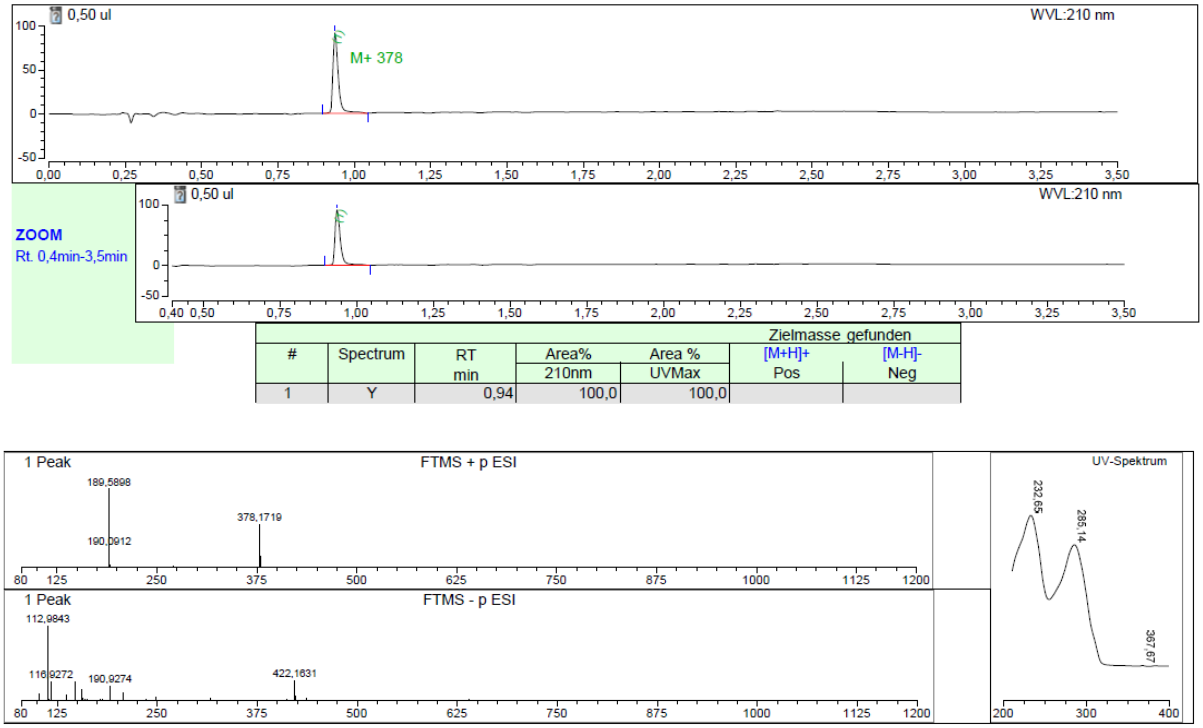
LC-MS (Method 2): Rt = 1.70 min; MS (ESIpos): m/z = 306 $[M+H]^+$

1-{(2Z)-3-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-2-fluoroprop-2-en-1-yl}-4-(methylamino)pyridinium formate (21):



5-[(1Z)-3-Chloro-2-fluoroprop-1-en-1-yl]-1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole (50 mg, 62 % purity, 0.1 mmol) was dissolved in 2 ml of DMF, potassium iodide (16.5 mg, 0.1 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (32.4 mg, 0.3 mmol) was added and the mixture was stirred at 50 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 12 mg (100 % purity, 28 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.94 min; MS (ESIpos): m/z = 378 $[M\text{-}HCOO]^+$

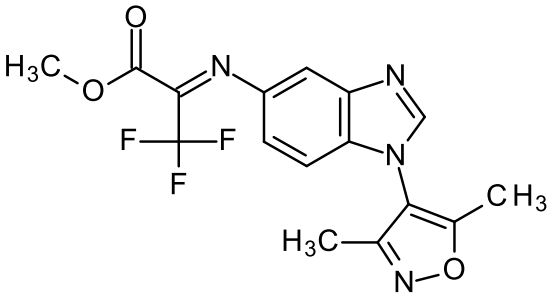


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,94	100,0	100,0		

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.01 (br s, 1H), 8.49 (s, 1H), 8.44-8.38 (m, 2H), 8.19 (d, J = 6.72 Hz, 1H), 7.92 (s, 1H), 7.48-7.39 (m, 2H), 6.95 (d, J = 7.46 Hz, 2H), 6.49-6.39 (m, 1H), 5.18 (d, J = 18.34 Hz, 2H), 2.92 (d, J 4.89 Hz, 3H), 2.33 (s, 3H), 2.10 (s, 3H).

<u>Preparation of (22) and respective starting materials:</u>

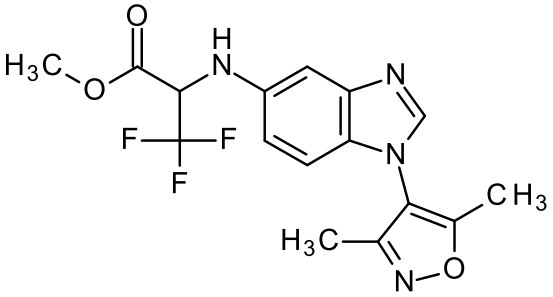
methyl (2Z)-2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]imino}-3,3,3-trifluoropropanoate:



1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-amine (250 mg, 1.10 mmol) was treated with 1.5 ml of toluene under argon. First methyl 3,3,3-trifluoro-2-oxopropanoate (0.12 ml, 1.10 mmol), then pyridine (0.18 ml, 2.19 mmol) was added, and finally thionyl chloride (80 µl, 1.10 mmol) was slowly added dropwise at room temperature. The mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product (70 % purity) was used in the next step without further purification.

LC-MS (Method 2): Rt = 1.76 min; MS (ESIpos): m/z = 367 $[M+H]^+$

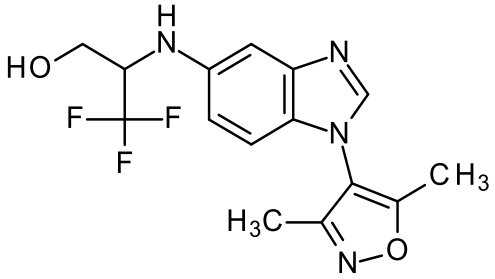
rac-methyl-N-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-3,3,3-trifluoroalaninate:



Zinc dust (143 mg, 2.19 mmol) was placed in 11 ml of acetic acid. Crude methyl (2Z)-2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]imino}-3,3,3-trifluoropropanoate (70 % purity) was added and the suspension was stirred at room temperature for 2 h. The reaction mixture was carefully neutralized with solid $NaHCO_3$ and extracted with ethyl acetate. The combined organic layers were washed with water, dried over sodium sulfate, filtered and evaporated. The crude product (534 mg, 88 % purity) was used in the next step without further purification.

LC-MS (Method 2): Rt = 1.48 min; MS (ESIpos): m/z = 369 $[M+H]^+$

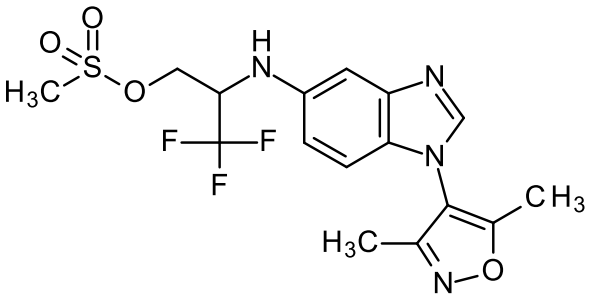
rac-2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-3,3,3-trifluoropropan-1-ol:



rac-Methyl-N-[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]-3,3,3-trifluoroalaninate (320 mg, 88 % purity, 0.77 mmol) was dissolved in 7 ml of THF. Lithium aluminum hydride solution (1 M, 0.77 ml, 0.77 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 1.5 h. The reaction mixture was quenched with some drops of concentrated NH_4Cl-solution, dried over sodium sulfate, filtered and evaporated. 221 mg (83 % purity, 71 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.11 min; MS (ESIpos): m/z = 341 $[M+H]^+$

rac-2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-3,3,3-trifluoropropyl methanesulfonate:

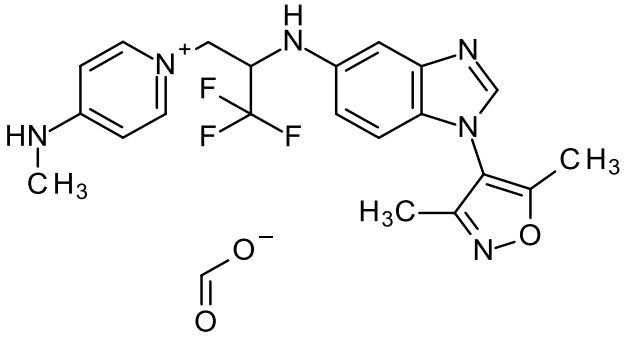


rac-2-{[1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-3,3,3-trifluoropropan-1-ol (221 mg, 83 % purity, 0.51 mmol) and DIPEA (0.18 ml, 1.01 mmol) were treated with 1 ml of dichloromethane. Methanesulfonyl chloride (60 µl, 0.76 mmol) was added dropwise to the reaction mixture at 0 °C. The

mixture was stirred at room temperature. After 2 h further DIPEA (90µl, 0.5 mmol) and methanesulfonyl chloride (20 µl, 0.25 mmol) were added and the mixture was stirred 2 h at room temperature. The reaction mixture was diluted with dichloromethane and washed with saturated $NaHCO_3$-solution. The combined organic layers were dried over sodium sulfate, filtered and evaporated. 263 mg (75 % purity, 87 % yield) of the title compound were obtained.

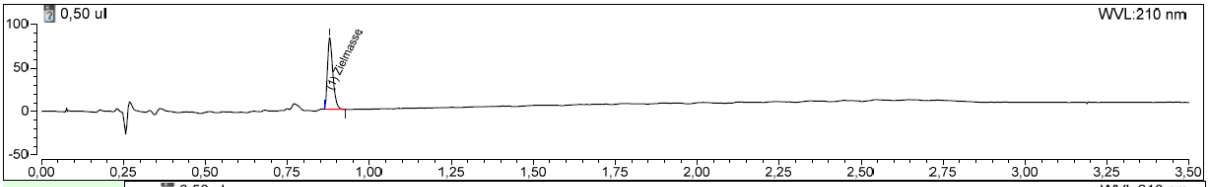
LC-MS (Method 2): Rt = 1.37 min; MS (ESIpos): m/z = 419 $[M+H]^+$

rac-1-(2-{[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-3,3,3-trifluoropropyl)-4-(methylamino)pyridinium formate (22):



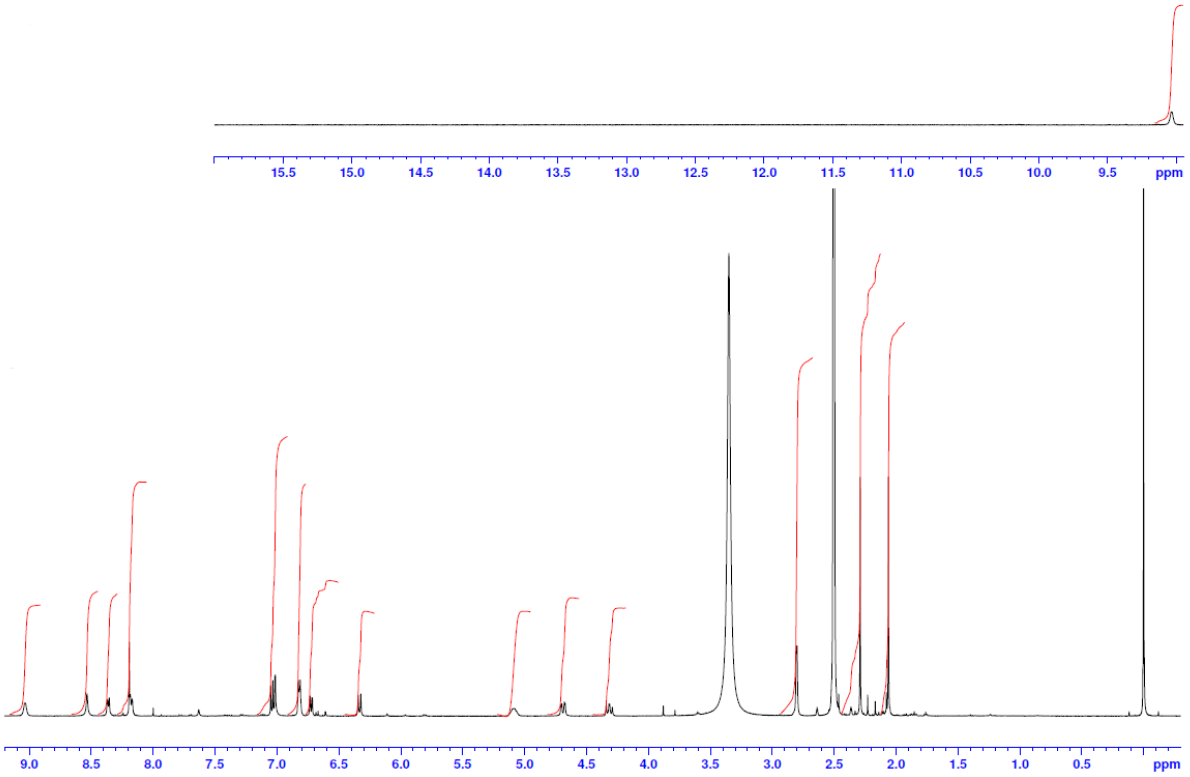
rac-2-{[1-(3,5-Dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]amino}-3,3,3-trifluoropropyl methanesulfonate (207 mg, 75 % purity, 0.37 mmol) was dissolved in 4.6 ml of DMF, potassium iodide (61.5 mg, 0.37 mmol) was added and the mixture was stirred at 50 °C for 30 min. Then N-methylpyridin-4-amine (120.4 mg, 1.11 mmol) and DIPEA (0.19 ml, 1.11 mmol) were added, and the mixture was stirred at 50 °C overnight. More N-methylpyridin-4-amine (120.4 mg, 1.11 mmol) and DIPEA (0.19 ml, 1.11 mmol) were added and the mixture was stirred at 75 °C overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18; eluent acetonitrile/water; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 0.8 mg (100 % purity, 0.4 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.88 min; MS (ESIpos): m/z = 431 $[M-HCOO]^+$



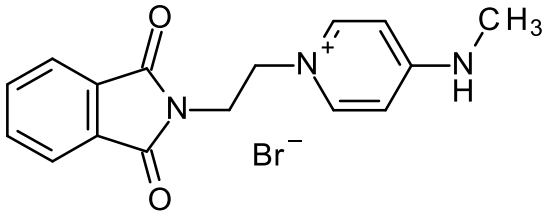
			Zielmasse gefunden			
#	Spectrum	RT min	Area% 210nm	Area % UVMax	[M]+ Pos	[M-H]- Neg
1	Y	0,88	100,0	100,0	431,1802	

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.03 (m, 1H), 8.54 (br s, 1H), 8.36 (d, J = 6.56 Hz, 1H), 8.19-8.17 (m, 2H), 7.05-7.01 (m, 2H), 6.82 (d, J = 7.48 Hz, 2H), 6.73 (dd, J = 8.7 Hz, 1H), 6.33 (d, J = 10.68 Hz, 1H), 5.13-5.05 (m, 1H), 4.67 (dd, J = 13.73 Hz, 1H), 4.30 (dd, J = 13.58 Hz, 1H), 2.80 (d, J = 3.05 Hz, 3H), 2.29 (s, 3H), 2.06 (s, 3H).



Preparation of (23) and respective starting materials:

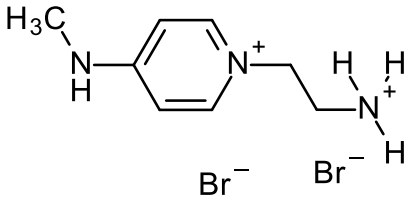
1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium bromide:



N-Methylpyridin-4-amine (500 mg, 4.62 mmol) was dissolved in 10 ml of DMF. 2-(2-bromoethyl)-1H-isoindole-1,3(2H)-dione (1.17 g, 4.62 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 1.43 g (97 % purity, 83 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.41 min; MS (ESIpos): m/z = 282 $[M-Br]^+$

1-(2-azaniumylethyl)-4-(methylamino)pyridinium dibromide:

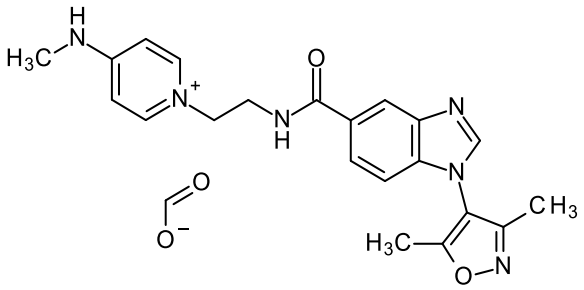


A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium bromide (1.43 g, 97 % purity, 3.83 mmol) in 5 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred overnight with THF. The solid was filtered off, washed THF and dried in vacuo. 1.15 g (100 % purity, 96 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.14 min; MS (ESIpos): m/z = 152 $[M-2Br-H]^+$

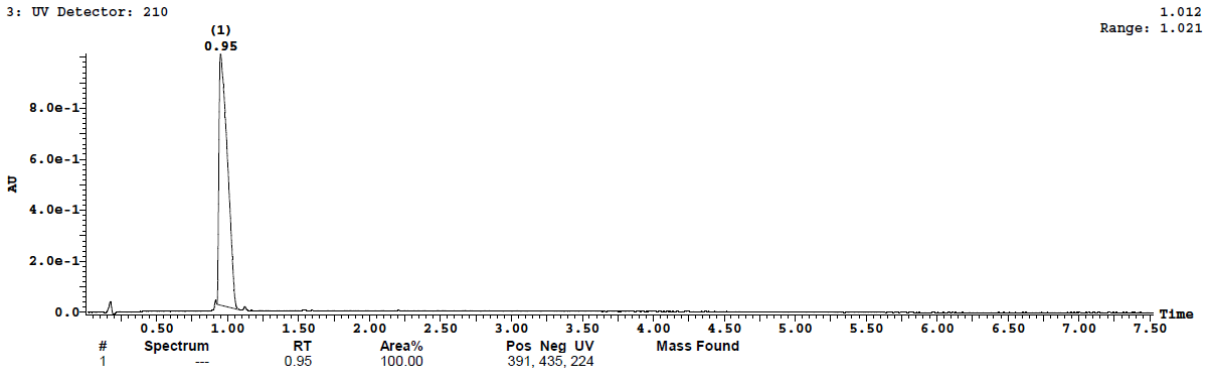
^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.79 (br d, J = 4.52 Hz, 1H), 8.31 (d, J = 7.21 Hz, 1H), 8.11 (d, J = 7.21 Hz, 1H), 8.03 (br s, 3H), 6.95 (m, 2H), 4.40 (t, J = 5.87 Hz, 2H), 3.37-3.30 (m, 2H), 2.92 (d, J = 5.01 Hz, 3H).

1-[2-({[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]carbonyl}amino)ethyl]-4-(methylamino) pyridinium formate (23):



1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazole-5-carboxylic acid (300 mg, 1.17 mmol) was dissolved in 10 ml of dichloromethane. 1-chloro-N,N,2-trimethylprop-1-en-1-amine (0.25 ml, 1.87 mmol) was added and the mixture was stirred at room temperature for 30 min. After that time pyridine (0.81 ml, 4.67 mmol) and 1-(2-azaniumylethyl)-4-(methylamino)pyridinium dibromide (365 mg, 1.17 mmol), solved in a mixture of 5 ml of dichloromethane and triethylamine (0.49 ml, 3.5 mmol) were added and the reaction was stirred at room temperature for 1h. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) two times. Product containing samples were united and the solvents were evaporated. 131 mg (100 % purity, 36 % yield) of the title compound were obtained.

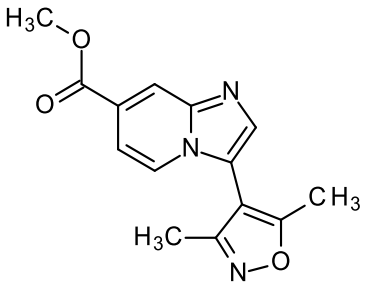
LC-MS (Method 3): Rt = 0.95 min; MS (ESIpos): m/z = 391 $[M-HCOO]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.11 (m, 1H), 8.88 (m, 1H), 8.53 (s, 2H), 8.32 (d, J 07.34 Hz, 1H), 8.26 (s, 1H), 8.11 (d, J = 7.09 Hz, 1H), 7.80 (d, J = 8.68 Hz 1H), 7.43 (d, J = 8.56 Hz, 1H), 6.89 (m, 1H), 6.84 (d, J = 7.21 Hz, 1H), 4.32 (t, J = 5.14 Hz, 2H), 3.72 (q, J = 5.46 Hz, 2H), 2.85 (d, J = 3.91 Hz, 3H), 2.33 (s, 3H), 2.10 (s, 3H).

Preparation of (24) and respective starting materials:

Methyl 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (39):

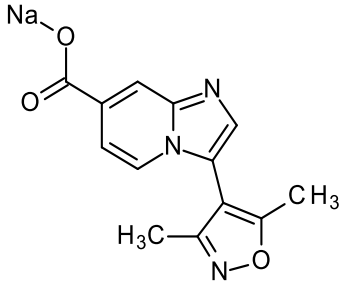


Methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (10 g, 33.11 mmol) was dissolved in 500 ml of DMF, (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (9.33 g, 66.21 mmol) and cesium fluoride (15.09 g, 99.31 mmol) were added to the solution and the mixture was degassed with argon for 10 min. Then [1,1-bis (diphenylphosphino)ferrocene]dichloropalladium (II) (1.35g, 1.66 mmol) was added and the mixture was stirred at 90 °C overnight. The precipitated solid was filtered off and discarded. The filtrate was concentrated to half, diluted with water and saturated $NaHCO_3$-solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, and evaporated. The crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP KP-Sil 100 g Ultra; eluent: Cy / EE: 12 % EE ->100 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 4.85 g (100 % purity, 54 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.09 min; MS (ESIpos): m/z = 272 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.28 (m, 1H), 8.24 (s, 1H), 7.95 (s, 1H), 7.35 (dd, J = 7.09 Hz, 1H), 3.91 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H).

Sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (40):

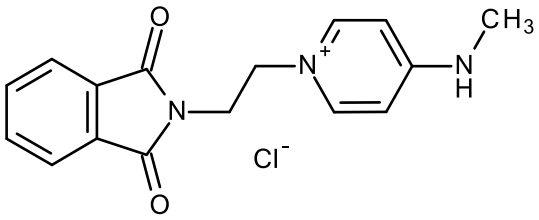


Methyl 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (3.94 g, 14.52 mmol) was dissolved in 80 ml of THF / MeOH 3:1. Sodium hydroxide solution (29.05 ml, 1M, 29.05 mmol) was added and the mixture was stirred at room temperature. After 30 min the reaction mixture was neutralized with 4N HCl and concentrated. The precipitate was stirred with methanol and insoluble solids were filtered off and discarded. The filtrate was concentrated, and the residue was stirred with acetonitrile. The solid was filtered off and dried in vacuo. 3.87 g (100 % purity, 95 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.54 min; MS (ESIpos): m/z = 258 $[M-Na+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.51 (br d, J = 6.36 Hz, 1H), 8.39 (s, 1H), 8.34 (s, 1H), 7.62 (br d, J = 6.97 Hz, 1H), 2.38 (s, 3H), 2.16 (s, 3H).

1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium chloride (42):

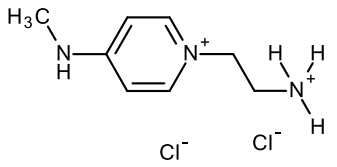


N-methylpyridin-4-amine (15.5 g, 143.11 mmol) was dissolved in 125 ml of DMF. 2-(2-chloroethyl)-1H-isoindole-1,3(2H)-dione (30.0 g, 143.11 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 28.6 g (100 % purity, 63 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.67 min; MS (ESIpos): m/z = 282 $[M\text{-}Cl]^+$

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.05 (m, 1H), 8.35 (dd, J = 7.41 Hz, 1H), 8.15 (dd, J = 7.33 Hz, 1H), 7.88-7.84 (m, 4H), 6.88 (dd, J = 7.29 Hz, 1H), 6.79 (dd, J = 7.41 Hz, 1H), 4.32 (m, 2H), 3.94 (m, 2H), 2.84 (d, J = 4.97 Hz, 3H).

1-(2-azaniumylethyl)-4-(methylamino)pyridinium dichloride (43):

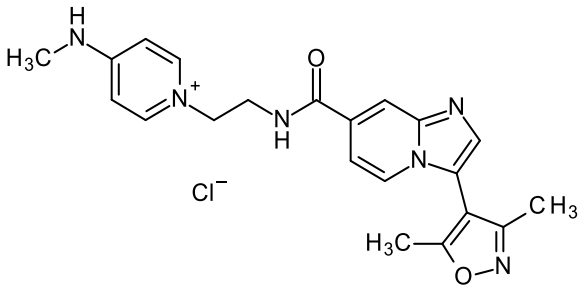


A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium chloride (22.9 g, 72.06 mmol) in 120 ml of conc. HCl was stirred at a bath temperature of 100 °C over the weekend. The reaction solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction through a frit (por. 3) and washed with water (2 × 20 ml). The filtrate was concentrated at a bath temperature of 70 °C on a rotary evaporator and then coevaporated twice with 250 ml of dichloromethane. The light brown residue was stirred overnight with 250 ml of THF. The solid was filtered off, washed two times with 50 ml of diisopropyl ether and dried in vacuo. 15.3 g (100 % purity, 95 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.14 min; MS (ESIpos): m/z = 152 $[M\text{-}2Cl\text{-}H]^+$

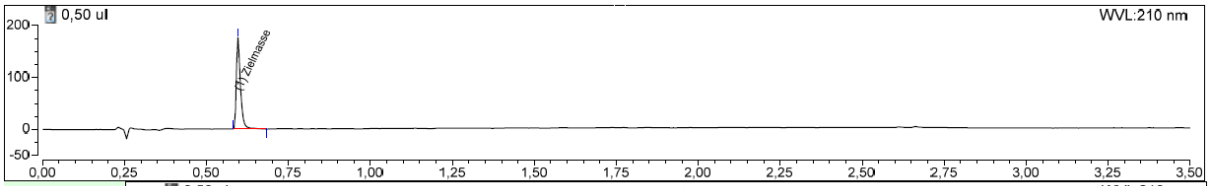
^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.13 (m, 1H), 8.63 (m, 3H), 8.39 (d, J = 7.21 Hz, 1H), 8.21 (d, J = 6.72 Hz, 1H), 7.00-6.91 (m, 2H), 4.42 (t, J = 5.75 Hz, 2H), 3.27 (m, 2H), 2.90 (d, J = 4.89 Hz, 3H).

1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium chloride (24):



1-(2-azaniumylethyl)-4-(methylamino)pyridinium dichloride (2.41 g, 10.74mmol), DMAP (3.94 g, 32.23 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.09 g, 16.12 mmol) were added to a solution of sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (3.0 g, 10.74 mmol) in 30 ml of dichloromethane and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP KP-NH 375 g; eluent: DCM / MeOH: 5 % MeOH ->40 % MeOH; flow: 150 ml/min). Product containing samples were united and the solvents were evaporated. 2.88 g (100 % purity, 63 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.60 min; MS (ESIpos): m/z = 391 $[M-Cl]^+$

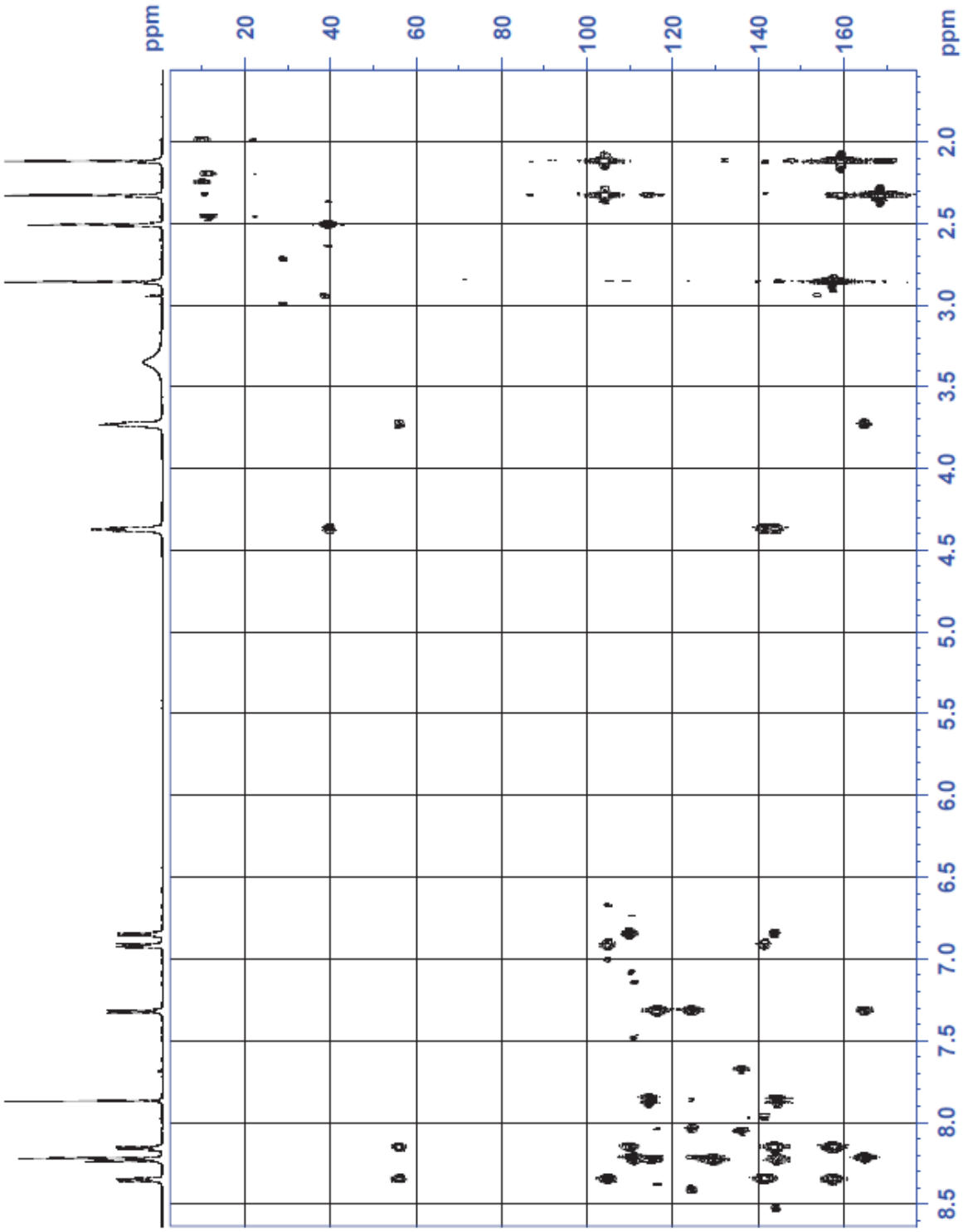


			Zielmasse gefunden			
#	Spectrum	RT	Area%	Area %	[M]+	[M-H]-
		min	210nm	UVMax	Pos	Neg
1	Y	0,60	100,0	100,0	391,1877	

HRMS: m/z$[M-Cl]^+$ calculated for C21H23N6O2: 391.1882, found: 391.1884.

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.10-8.77 (m, 2H), 8.34 (d, J = 7.34 Hz, 1H), 8.21 (m, 2H), 8.10 (d, J = 6.97 Hz, 1H), 7.87 (s, 1H), 7.29 (d, J = 7.09 Hz, 1H), 6.86 (m, 2H), 4.34 (m, 2H), 3.65 (m, 2H), 2.86 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H).

^{13}C-NMR (126 MHz, DMSO-d6): δ [ppm] = 168.4 (C), 164.9 (C), 159,3 (C), 157.4 (C), 144.5 (C), 143.9 (CH), 141,4 (CH), 136.1 (CH), 129.5 (C), 124.5 (CH), 116.4 (CH), 114.4 (CH), 110.9 (CH), 110.0 (CH), 106.6 (C), 104.8 (CH), 56.1 (CH2), 40.0 (CH2), 28.9 (CH3), 11.5 (CH3), 10.0 (CH3).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120
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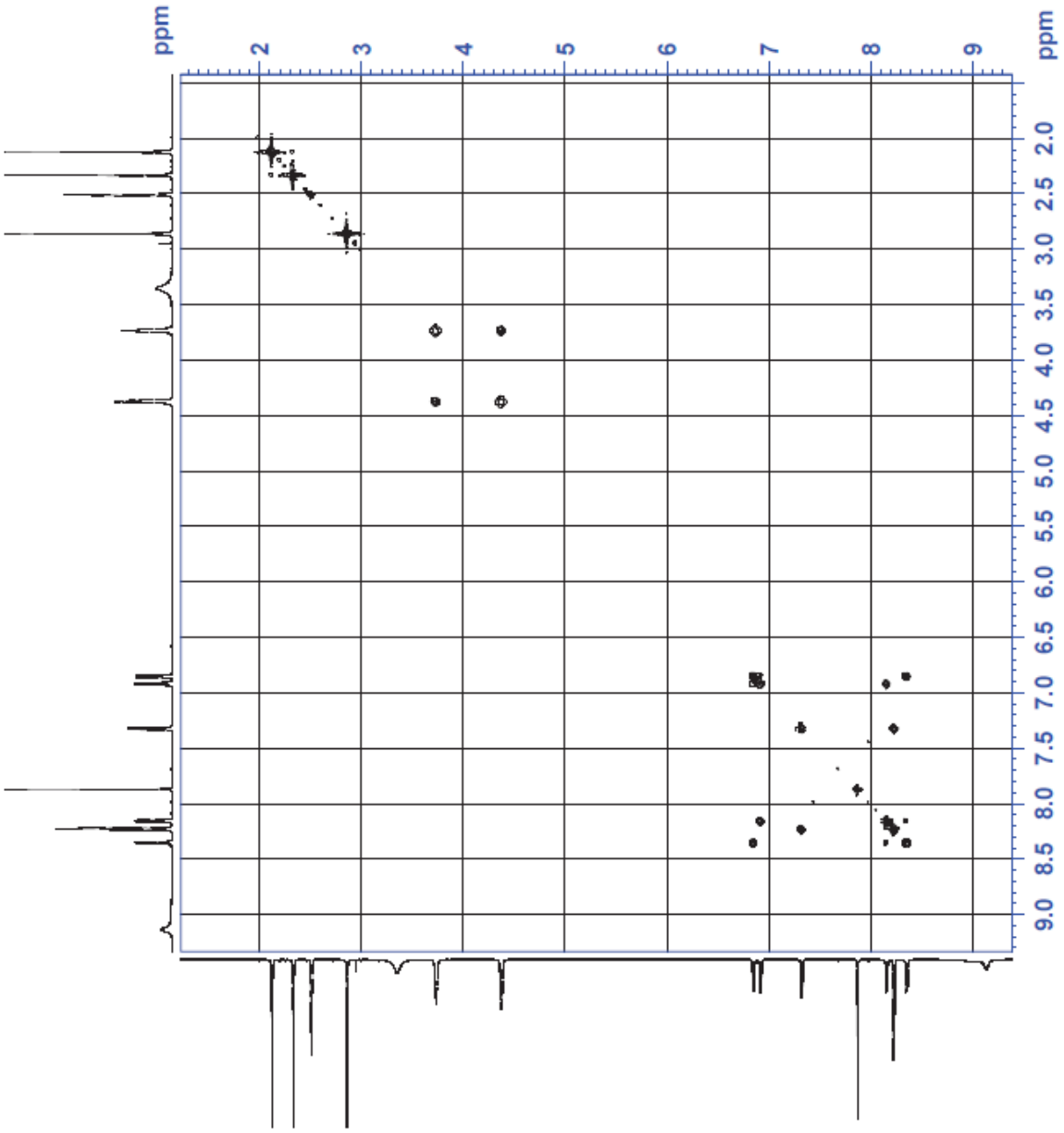
8.5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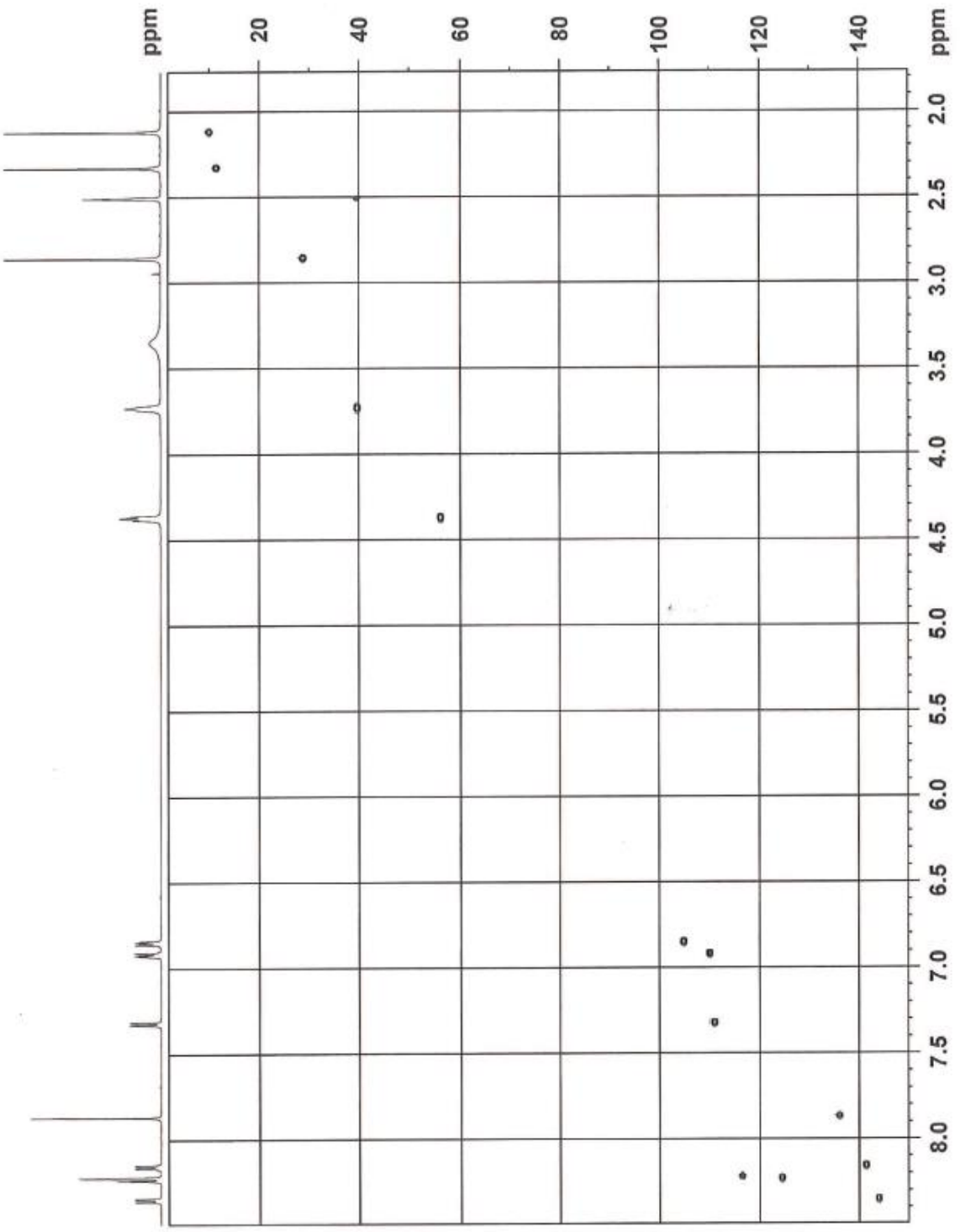
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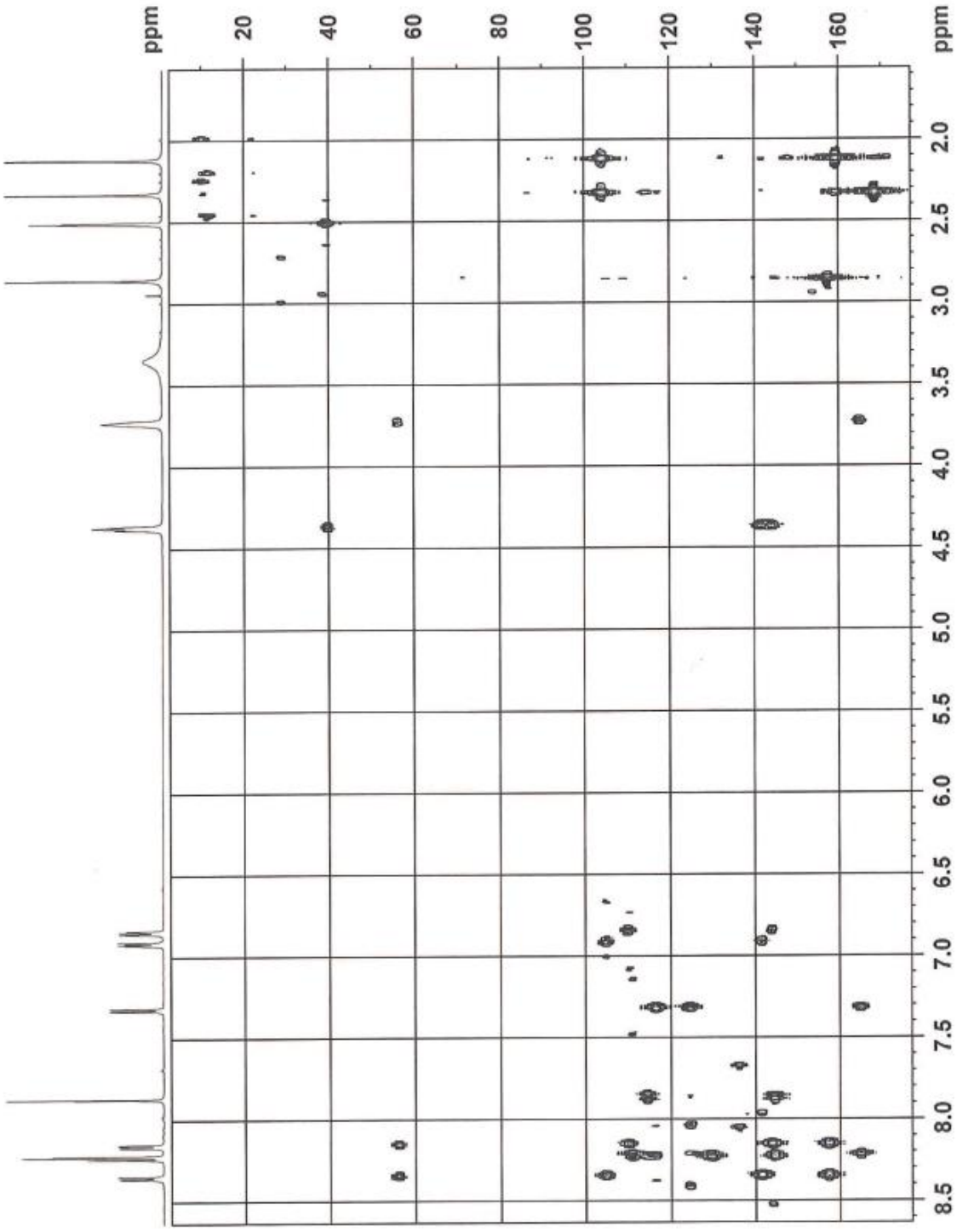
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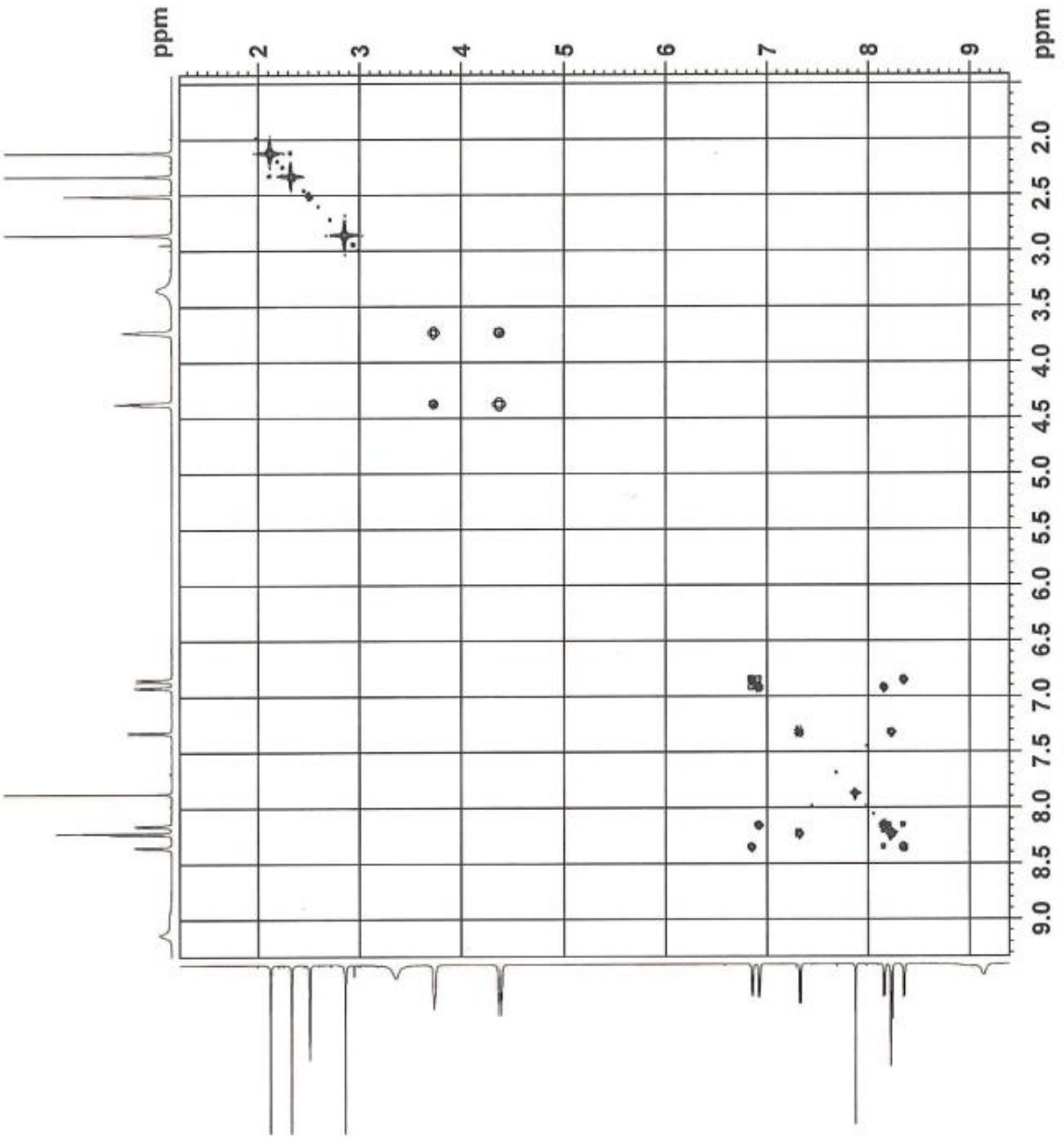
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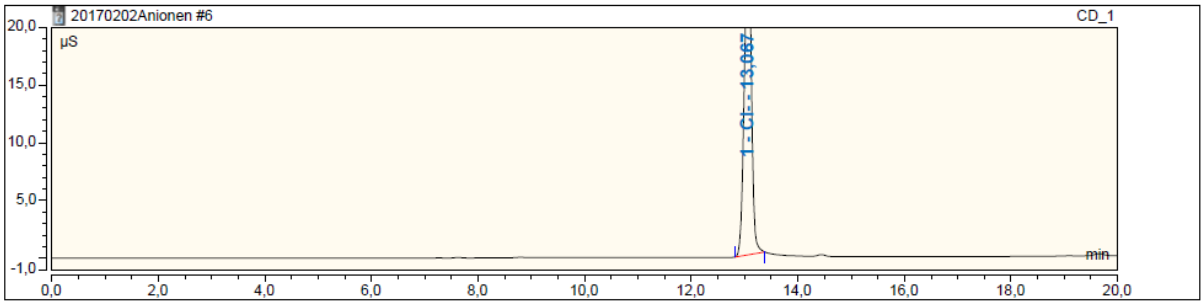
8.0
8.5
9.0

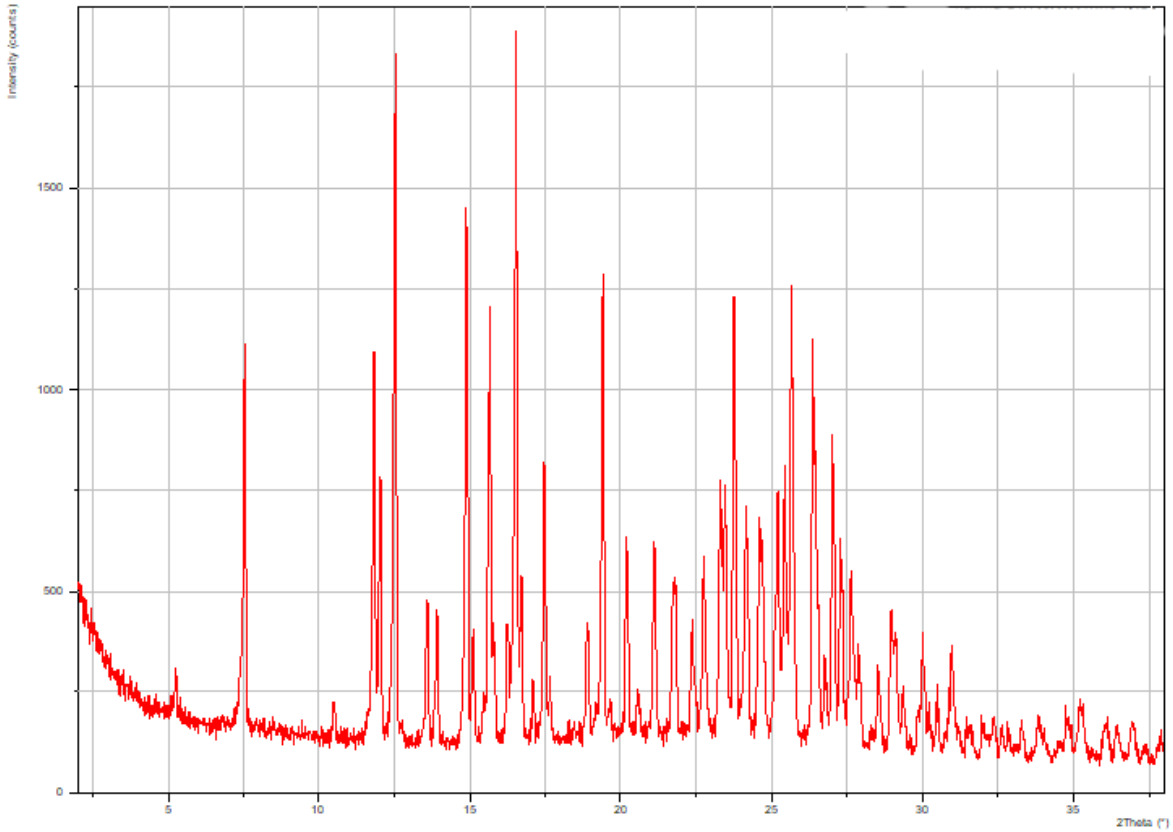


No.	Name	Time min	Massenanteil in (Gew. %) Cl-
2	Leer	n.a.	n.a.
5	P1	12,89	7,9
6	P2	13,07	7,6
	Average:	12,98	7,7

Ion chromatography: w(Cl-) = 7.7 weight % = 1 eq. (Cl-).

Preparation of crystalline material 1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium chloride (24):

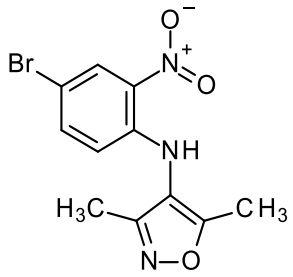
4.53 g of amorphous 1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium chloride were stirred in 176 ml of acetone / 2-propanol (60:40) at 25 °C for one week. 3.95 g product was obtained after filtration and drying in vacuo. According to powder diffraction the compound is crystalline:



The melting point of the crystalline compound is 239°C.

Preparation of (25) and respective starting materials:

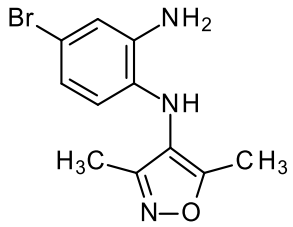
N-(4-bromo-2-nitrophenyl)-3,5-dimethyl-1,2-oxazol-4-amine:



4-Bromo-1-fluoro-2-nitrobenzene (5 g, 22.73 mmol) was dissolved in 50 ml of NMP. 3,5-dimethyl-1,2-oxazol-4-amine (2.8 g, 25 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 340 g; eluent: Cy / EE: 2 % EE -> 20 % EE; flow: 200 ml/min). Product containing samples were united and the solvents were evaporated. 2.86 g (100 % purity, 40 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.98 min; MS (ESIpos): m/z = 311 $[M+H]^+$

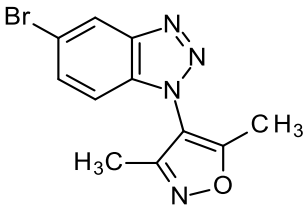
4-bromo-N1-(3,5-dimethyl-1,2-oxazol-4-yl)benzene-1,2-diamine:



N-(4-Bromo-2-nitrophenyl)-3,5-dimethyl-1,2-oxazol-4-amine (2.86 g, 9.15 mmol) was dissolved in 36 ml of acetic acid. Tin(II)chloride dihydrate (10.32 g, 45.75 mmol) dissolved in 36 ml of conc. hydrochloric acid was added dropwise and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured onto ice water, basified with 50 % sodium hydroxide solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: Cy / EE: 12 % EE -> 100 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 2.22 g (100 % purity, 86 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.67 min; MS (ESIpos): m/z = 282 $[M+H]^+$

5-bromo-1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzotriazole:

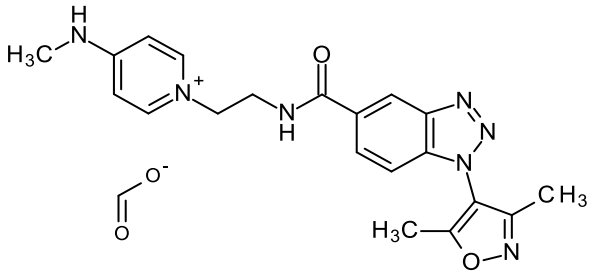


4-Bromo-N1-(3,5-dimethyl-1,2-oxazol-4-yl)benzene-1,2-diamine (2.2 g, 7.85 mmol) was dissolved in 20 ml of DMSO. 20 ml of sulfuric acid (30 %) were added, and the mixture was cooled to 0 °C. A solution of sodium nitrite (596 mg, 8.64 mmol) in 10 ml of water was added dropwise at this temperature. The

mixture was stirred for 20 minutes at 0 °C before a solution of sodium iodide (3.71 g, 24.73 mmol) in 10 ml of water was added at 0 °C dropwise. The ice bath was removed, and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was poured onto ice water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: Cy / EE: 5 % EE -> 40 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 1.84 g (100 % purity, 80 % yield) of the title compound were obtained.

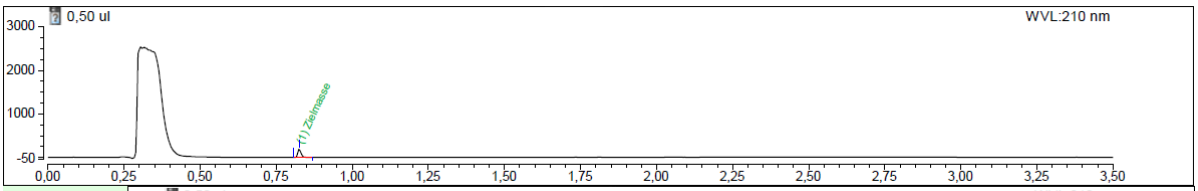
LC-MS (Method 2): Rt = 1.81 min; MS (ESIpos): m/z = 293 $[M+H]^+$

1-[2-({[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzotriazol-5-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium bromide (25):



The reaction was carried out in a COware screwable two-chamber glass system. 5-bromo-1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzotriazole (33 mg, 0.11 mmol), 1-(2-aminoethyl)-4-(methylamino)pyridinium bromide hydrobromide (35 mg, 0.11 mmol), triethylamine (47 µl, 0.34 mmol), 4,5-Bis-(diphenylphosphino)-9,9-dimethyl xanthene (6.5 mg, 0.01 mmol) and bis (dibenzylideneacetone) palladium (0) (3.2 mg, 0.01 mmol) were dissolved in 2.5 ml of DMF under argon in the first chamber. In the second chamber, formic acid (21 µl, 0.56 mmol) was added dropwise to conc. sulfuric acid (3.28 ml, 61.49 mmol). The reaction mixture was then stirred for 1.5 h at 60 °C. and the crude product was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 1.5 mg (100 % purity, 3 % % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.82 min; MS (ESIpos): m/z = 391 $[M-HCOO]^+$

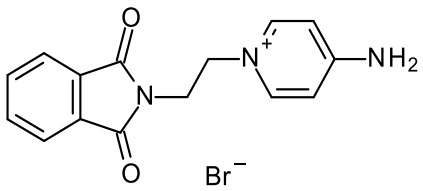


			Zielmasse gefunden			
#	Spectrum	RT	Area%	Area %	[M]+	[M-H]-
		min	210nm	UVMax	Pos	Neg
1	Y	0,82	100,0	100,0	392,1830	

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 8.98 (m, 1H), 8.72 (m, 1H), 8.65 (s, 1H), 8.50 (m, 1H), 8.32 (d, J = 6.94 Hz, 1H), 8.13 (d, J = 7.49 Hz, 1H), 8.03 (dd, J = 8.71 Hz, 1H), 7.81 (d, J = 8.67 Hz, 1H), 6.85 (m, 2H), 4.33 (t, J = 5.12 Hz, 2H), 3.75 (q, J = 5.25 Hz, 2H), 2.86 (d, J = 4.89 Hz, 3H), 2.40 (s, 3H), 2.15 (s, 3H).

Preparation of (26) and respective starting materials:

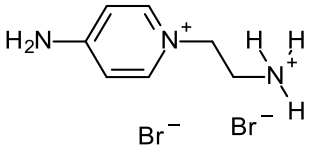
4-amino-1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]pyridinium bromide:



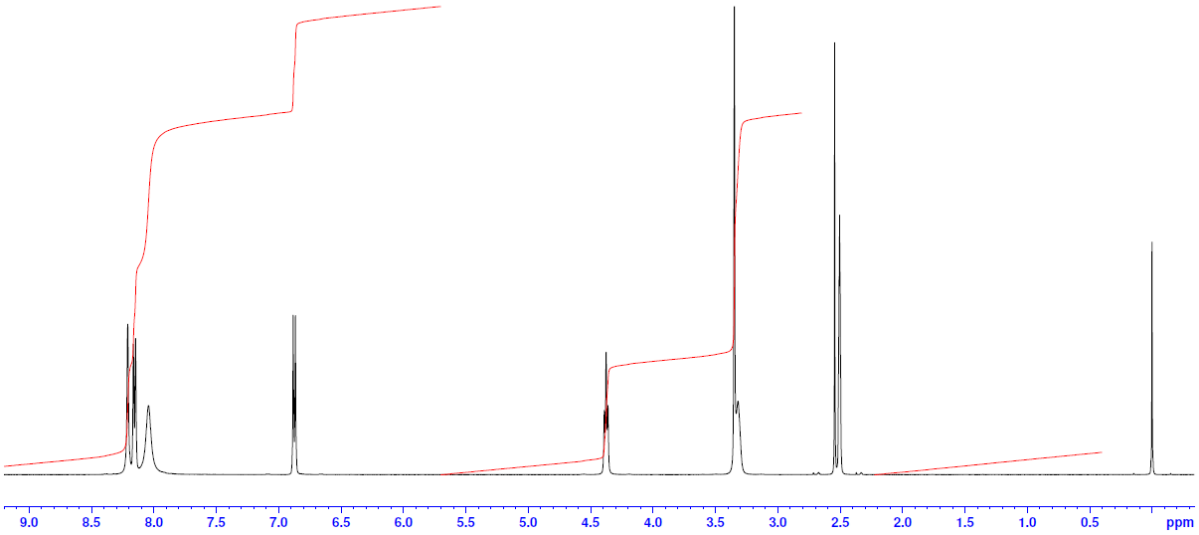
Pyridin-4-amine (2.0 g, 21.25 mmol) was dissolved in 20 ml of DMF. 2-(2-bromoethyl)-1H-isoindole-1,3(2H)-dione (5.4 g, 21.25 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 6.22 g (100 % purity, 84 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.63 min; MS (ESIpos): m/z = 268 $[M-Br]^+$

4-amino-1-(2-azaniumylethyl)pyridinium dibromide:

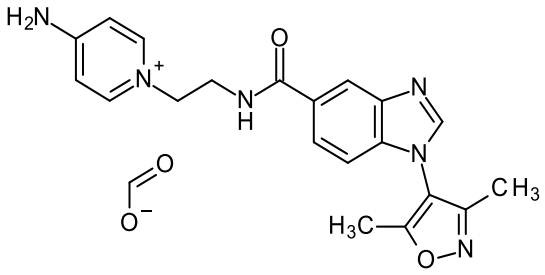


A solution of 4-amino-1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]pyridinium bromide (6.22 g, 17.86 mmol) in 25 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 4.26 g (100 % purity, 80 % yield) of the title compound were obtained.



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.21 (s, 2H), 8.16 (d, J = 7.09 Hz, 2H), 8.04 (s, 3H), 6.87 (d, J = 7.34 Hz, 2H), 4.36 (t, J = 5.93 Hz, 2H), 3.31 (m, 2H).

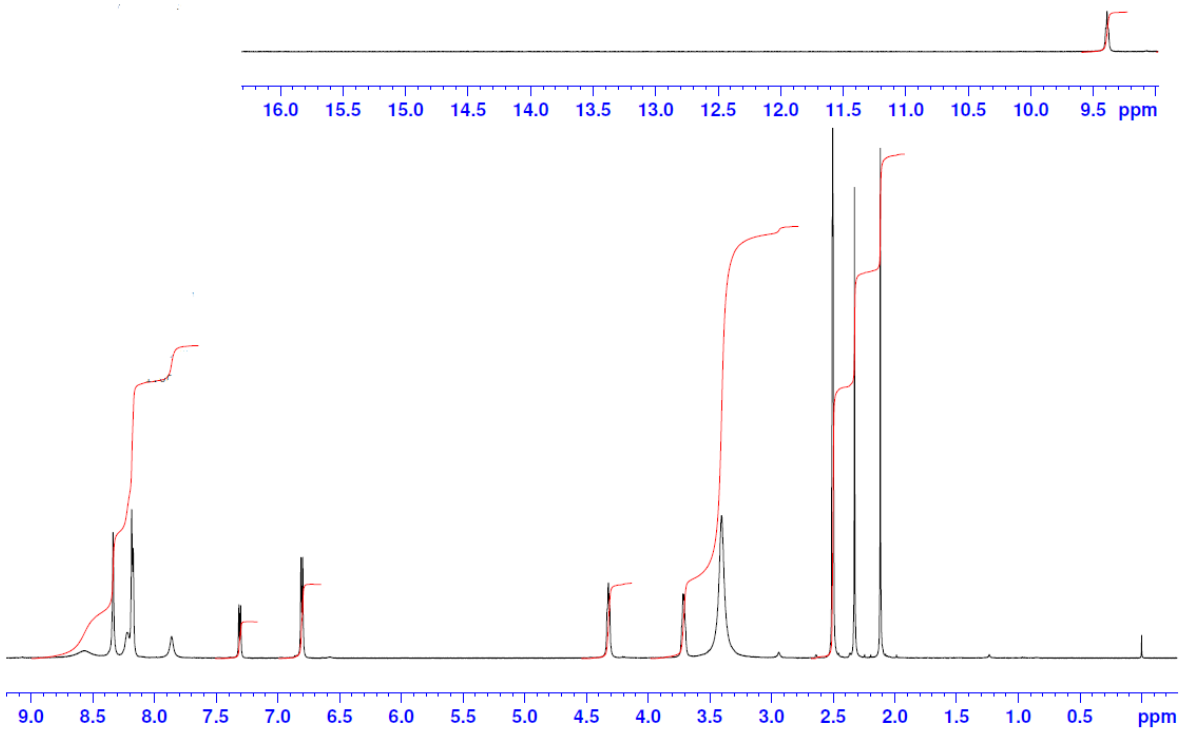
4-amino-1-[2-({[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]carbonyl}amino)ethyl]pyridinium formate (26):



4-Amino-1-(2-azaniumylethyl)pyridinium dibromide (58 mg, 0.19 mmol), DMAP (71 mg, 0.58 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (56 mg, 0.29 mmol) were added to a solution of sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (50 mg, 0.19 mmol) in 5 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) Product containing samples were united and the solvents were evaporated. 7 mg (94 % purity, 8 % yield) of the title compound were obtained.

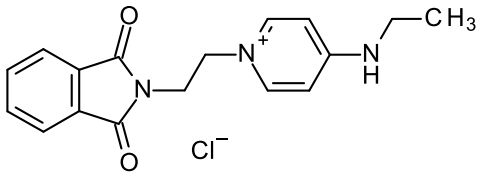
LC-MS (Method 1): Rt = 0.28 min; MS (ESIpos): m/z = 377 $[M\text{-}HCOO]^+$

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.38 (t, J = 4.89 Hz, 1H), 8.69-8.46 (m, 1H), 8.34 (s, 2H), 8.26-8.14 (m, 4H), 7.86 (s, 1H), 7.32 (d, J = 7.09 Hz, 1H), 6.80 (d, J = 7.01 Hz, 2H), 4.32 (t, J = 4.81 Hz, 2H), 3.71 (q, J = 4.65, 2H), 2.33 (s, 3H), 2.12 (s, 3H).



Preparation of (27) and respective starting materials:

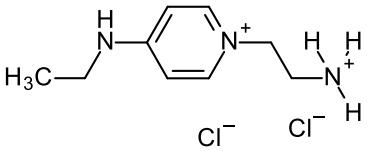
1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(ethylamino)pyridinium chloride:



N-Ethylpyridin-4-amine (500 mg, 4.09 mmol) was dissolved in 10 ml of DMF. 2-(2-chloroethyl)-1H-isoindole-1,3(2H)-dione (5.4 g, 21.25 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 849 mg (100 % purity, 63 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.77 min; MS (ESIpos): m/z = 296 $[M-Cl]^+$

1-(2-azaniumylethyl)-4-(ethylamino)pyridinium dichloride:

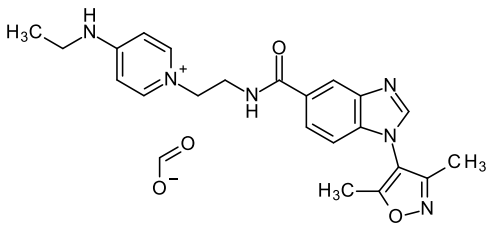


A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(ethylamino)pyridinium chloride (849 mg, 2.56 mmol) in 5 ml of conc. HCl was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 543 mg (100 % purity, 89 % yield) of the title compound were obtained.

LC-MS (Method 4): Rt = 1.04 min; MS (ESIpos): m/z = 166 $[M-2Cl]^+$

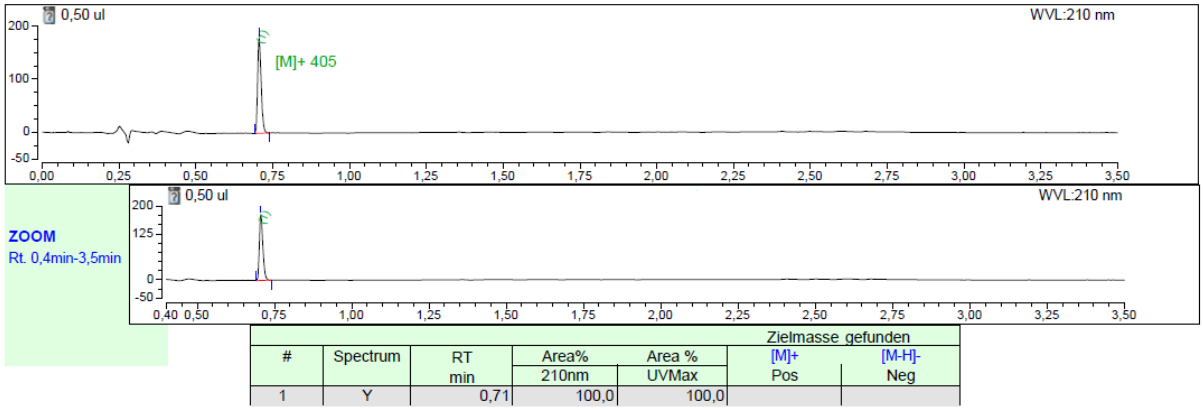
^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.87 (m, 1H), 8.41-8.24 (m, 4H), 8.11 (m, 1H), 6.97 (d, J = 7.46 Hz, 1H), 6.90 (d, J = 7.15 Hz, 1H), 4.42 (m, 2H), 3.36-3.27 (m, 4H), 1.19 (t, J = 7.21 Hz, 3H).

1-[2-({[1-(3,5-dimethyl-1,2-oxazol-4-yl)-1H-benzimidazol-5-yl]carbonyl}amino)ethyl]-4-(ethylamino)pyridinium formate (27):

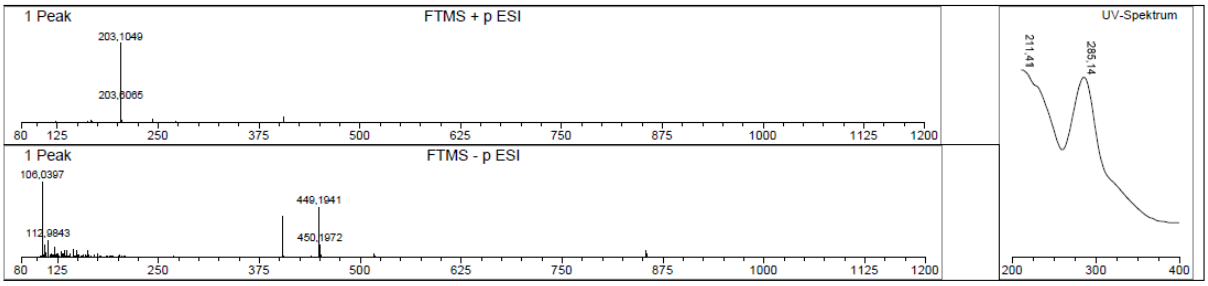


1-(2-Azaniumylethyl)-4-(ethylamino)pyridinium dichloride (93 mg, 0.36 mmol), DMAP (131 mg, 1.07 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (103 mg, 0.54 mmol) were added to a solution of sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (100 mg, 0.36 mmol) in 2 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) Product containing samples were united and the solvents were evaporated. 112 mg (100 % purity, 69 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.71 min; MS (ESIpos): m/z = 405 $[M\text{-}HCOO]^+$



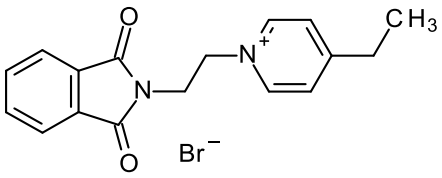
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,71	100,0	100,0		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.28-9.14 (m, 1H), 9.04-8.90 (m, 1H), 8.55 (s, 1H), 8.29 (d, J = 6.36 Hz, 1H), 8.23 (d, J = 7.09 Hz, 1H), 8.17 (s, 1H), 8.12 (d, J = 7.46 Hz, 1H), 7.86 (s, 1H), 7.29 (d, J = 7.09 Hz, 1H), 6.86 (m, 2H), 4.30 (t, J = 4.89 Hz, 2H), 3.71 (m, 2H), 3.25 (m, 2H), 2.32 (s, 3H), 2.12 (s, 3H), 1.15 (t, J = 7.21 Hz, 3H).

<u>Preparation of (28) and respective starting materials:</u>

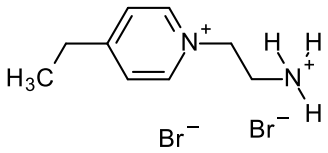
1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-ethylpyridinium bromide:



4-Ethylpyridin (500 mg, 4.67 mmol) was dissolved in 10 ml of DMF. 2-(2-bromoethyl)-1H-isoindole-1,3(2H)-dione (51.2 g, 4.67 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 1.35 g (85 % purity, 68 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.44 min; MS (ESIpos): m/z = 281 $[M\text{-}Br]^+$

1-(2-azaniumylethyl)-4-ethylpyridinium dibromide:

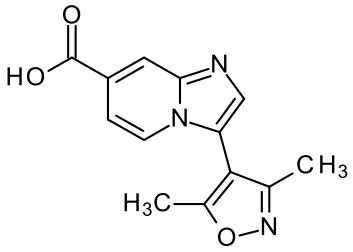


A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-ethylpyridinium bromide (1.35 g, 85 % purity, 3.18 mmol) in 20 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 1.11 g (80 % purity, 89 % yield) of the title compound were obtained.

LC-MS (Method 4): Rt = 0.23 min; MS (ESIpos): m/z = 152 $[M-Br]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.92 (d, J = 6.36 Hz, 2H), 8.10 (d, J = 6.72 Hz, 2H), 8.08-7.98 (m, 3H), 4.80 (t, J = 5.69 Hz, 2H), 3.51 (m, 2H), 2.94 (q, J = 7.58 Hz, 2H), 1.28 (t, J = 7.52 Hz, 3H).

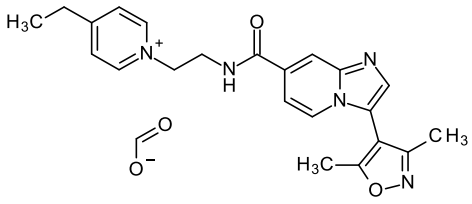
3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid:



In an argon atmosphere methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (2 g, 6.62 mmol) and tetrakis (triphenylphosphine) palladium (0) (383 mg, 0.33 mmol) were dissolved in 53 ml of DME. (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (2.33 g, 16.55 mmol), potassium carbonate (1.83 g, 13.24 mmol) and 26 ml of water were added and the mixture was stirred at 75 °C for 48 h. The reaction mixture was acidified with 1 N HCl and the solvents were removed on a rotary evaporator. The residue was suspended in dichloromethane / methanol 1:1, the insoluble solid was filtered off and the filtrate was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 5 % MeOH -> 40 % MeOH; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 790 mg (100 % purity, 46 % yield) of the title compound were obtained.

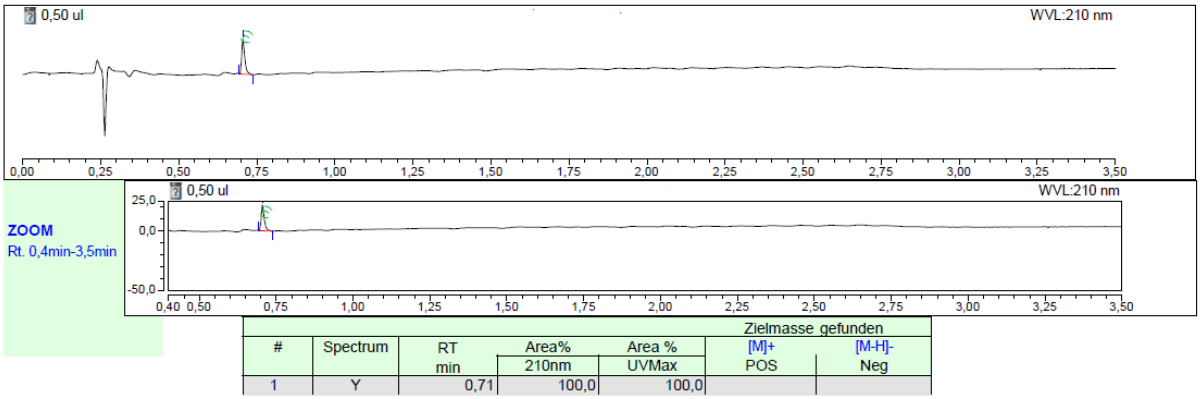
LC-MS (Method 5): Rt = 0.31 min; MS (ESIpos): m/z = 258 $[M+H]^+$

1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-ethylpyridinium formate (28):

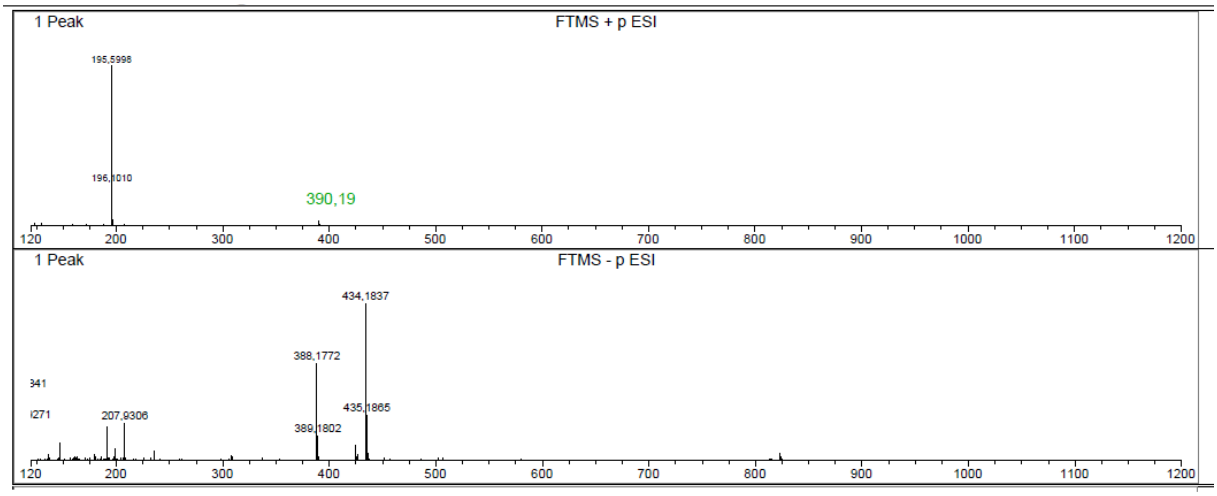


1-(2-Azaniumylethyl)-4-ethylpyridinium dibromide (61 mg, 80 % purity, 0.15 mmol), DMAP (95 mg, 0.78 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (56 mg, 0.29 mmol) were added to a solution of 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid (50 mg, 0.19 mmol) in 2 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) Product containing samples were united and the solvents were evaporated. 22 mg (100 % purity, 26 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.71 min; MS (ESIpos): m/z = 390 $[M\text{-}HCOO]^+$



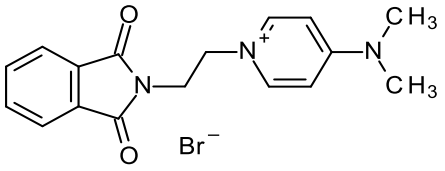
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ POS	[M-H]- Neg
1	Y	0,71	100,0	100,0		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.47-9.21 (m, 1H), 8.99 (br d, J = 5.38 Hz, 2H), 8.48 (s, 1H), 8.22 (d, J = 7.09 Hz, 1H), 8.15 (s, 1H), 8.00 (d, J = 6.24 Hz, 2H), 7.86 (s, 1H), 7.21 (d, J = 7.09 Hz, 1H), 4.73 (br. s, 2H), 3.87 (m, 2H), 2,89 (q, J = 7.7 Hz, 2H), 2,32 (s, 3H), 2,11 (s, 3H), 1.24 (t, J = 7.52 Hz, 3H).

<u>Preparation of (29) and respective starting materials:</u>

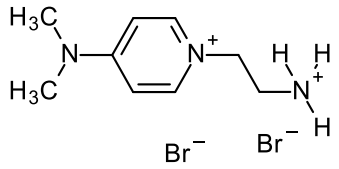
4-(dimethylamino)-1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]pyridinium bromide:



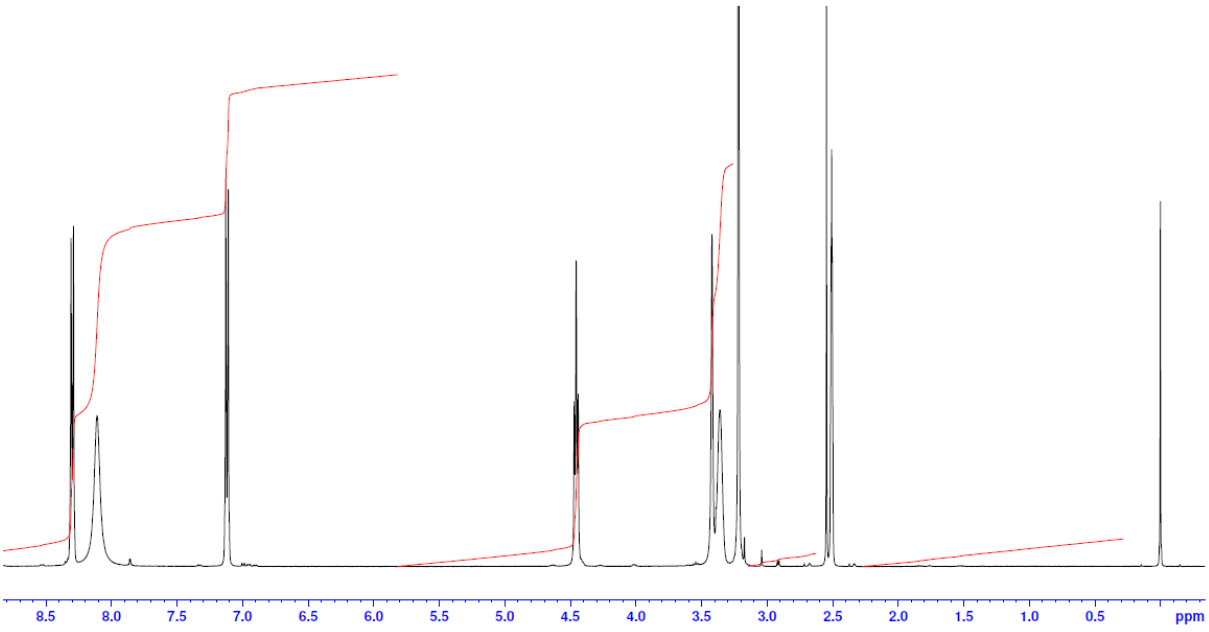
N,N-Dimethylpyridin-4-amine (2.0 g, 16.37 mmol) was dissolved in 20 ml of DMF. 2-(2-bromoethyl)-1H-isoindole-1,3(2H)-dione (4.16 g, 16.37 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 5.04 g (100 % purity, 82 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.75 min; MS (ESIpos): m/z = 296 $[M\text{-}Br]^+$

1-(2-azaniumylethyl)-4-(dimethylamino)pyridinium dibromide:

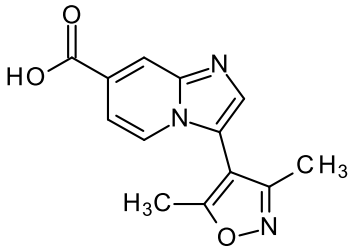


A solution of 4-(dimethylamino)-1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]pyridinium bromide (5.04 g, 13.40 mmol) in 19 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 3.55 g (100 % purity, 81 % yield) of the title compound were obtained.



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.33 (d, J = 7.7 Hz, 2H), 8.12 (m, 3H), 7.11 (d, J = 7.7 Hz, 2H), 4.44 (t, J = 5.93 Hz, 2H), 3.44 (m, 2H), 3.22 (s, 6H).

3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid:

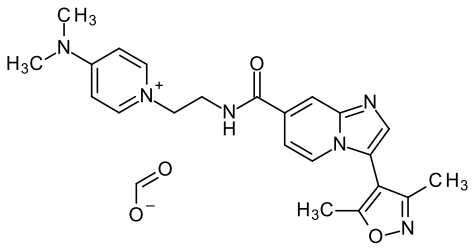


In an argon atmosphere methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (2 g, 6.62 mmol) and tetrakis (triphenylphosphine) palladium (0) (383 mg, 0.33 mmol) were dissolved in 53 ml of DME. (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (2.33 g, 16.55 mmol), potassium carbonate (1.83 g, 13.24 mmol) and 26 ml of water were added and the mixture was stirred at 75 °C for 48 h. The reaction mixture was acidified with 1 N HCl and the solvents were removed on a rotary evaporator. The residue was suspended in dichloromethane / methanol 1:1, the insoluble solid was filtered off and the filtrate was purified by column

chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 5 % MeOH -> 40 % MeOH; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 790 mg (100 % purity, 46 % yield) of the title compound were obtained.

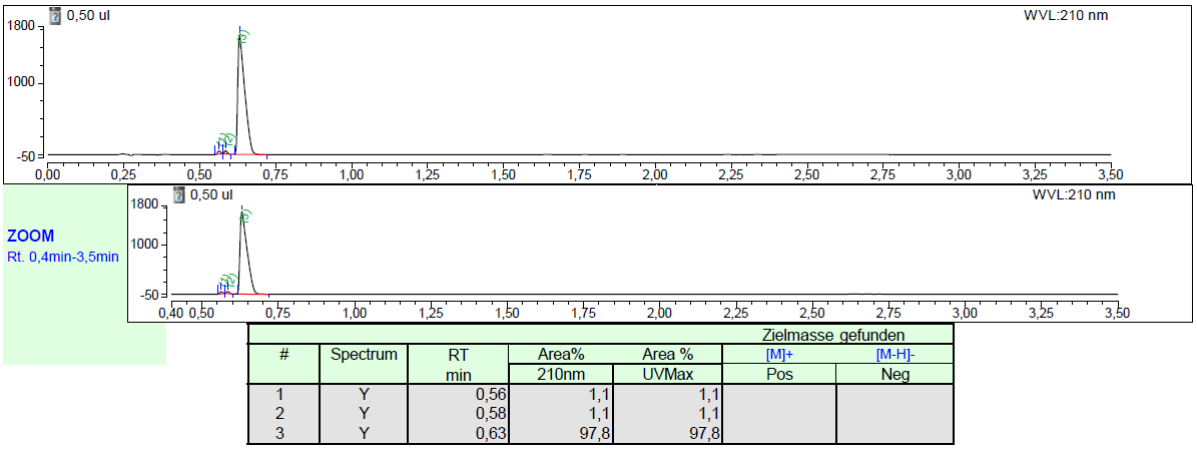
LC-MS (Method 5): Rt = 0.31 min; MS (ESIpos): m/z = 258 $[M+H]^+$

4-(dimethylamino)-1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]pyridinium formate (29):

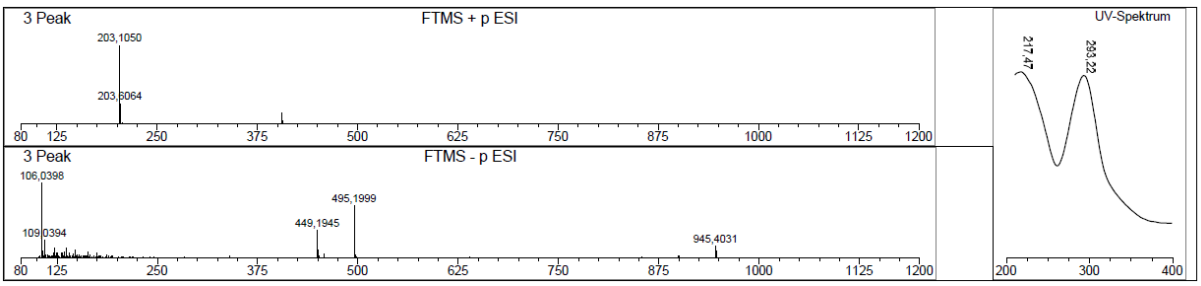


1-(2-Azaniumylethyl)-4-(dimethylamino)pyridinium dibromide (64 mg, 0.19 mmol), DMAP (71 mg, 0.58 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (56 mg, 0.29 mmol) were added to a solution of 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid (50 mg, 0.19 mmol) in 5 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) Product containing samples were united and the solvents were evaporated. 50 mg (98 % purity, 56 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.61 min; MS (ESIpos): m/z = 405 $[M-HCOO]^+$



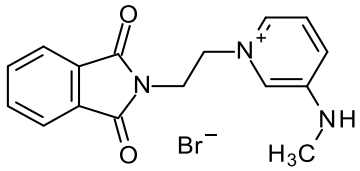
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,56	1,1	1,1		
2	Y	0,58	1,1	1,1		
3	Y	0,63	97,8	97,8		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.57-9.30 (m, 1H), 8.50 (m, 1H), 8.34 (m, 2H), 8.21 (m, 2H), 7.85 (s, 1H), 7.31 (d, J = 7.09 Hz, 1H), 7.01 (d, J = 6.97 Hz, 2H), 4.41 (br. s, 2H), 3.75 (q, J = 4.77 Hz, 2H), 3.16 (s, 6H), 2,32 (s, 3H), 2,11 (s, 3H).

Preparation of (30) and respective starting materials:

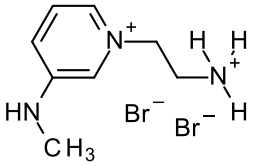
1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-3-(methylamino)pyridinium bromide:



N-Methylpyridin-3-amine (1.0 g, 9.25 mmol) was dissolved in 20 ml of DMF. 2-(2-bromoethyl)-1H-isoindole-1,3(2H)-dione (2.35 g, 9.25 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The solvent was removed at a rotary evaporator and the residue was stirred with dichloromethane. The solid was filtered off, washed with dichloromethane, and dried in vacuo. 1.8 g (100 % purity, 54 % yield) of the title compound were obtained.

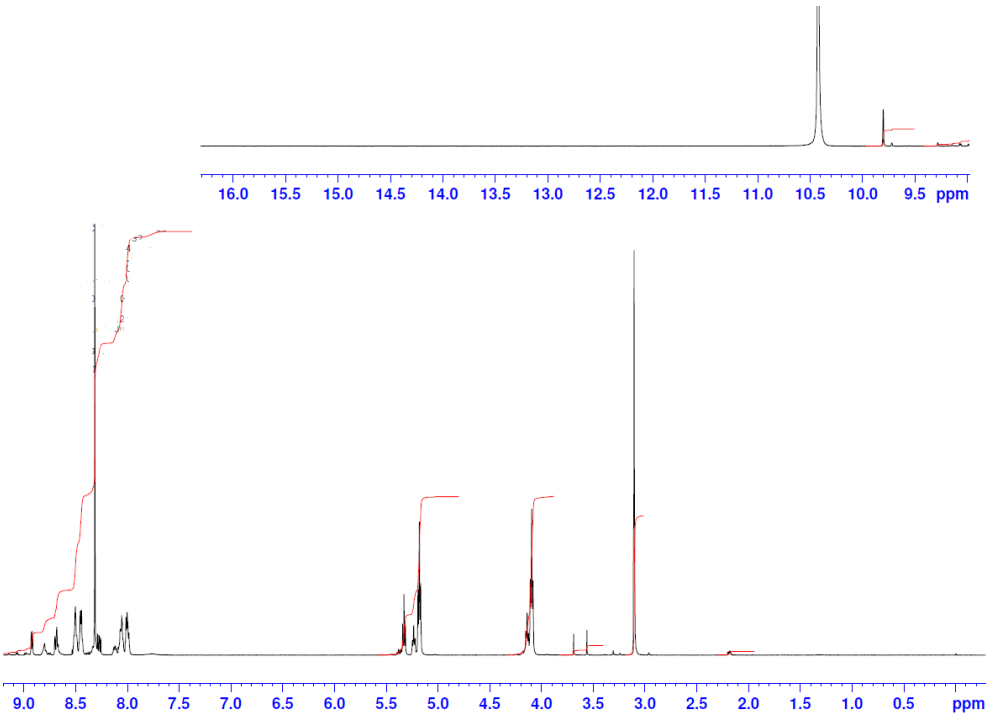
LC-MS (Method 2): Rt = 0685 min; MS (ESIpos): m/z = 282 $[M-Br]^+$

1-(2-azaniumylethyl)-3-(methylamino)pyridinium dibromide:

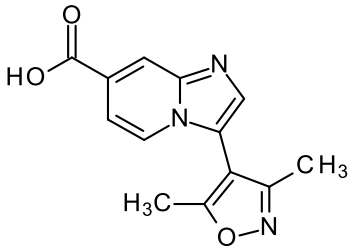


A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-3-(methylamino)pyridinium bromide (1.80 g, 4.97 mmol) in 6.6 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated on a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 1.7 g (75 % purity, 82 % yield) of the title compound were obtained.

^{1}H-NMR (500 MHz, formic-acid-d_2) δ [ppm]:



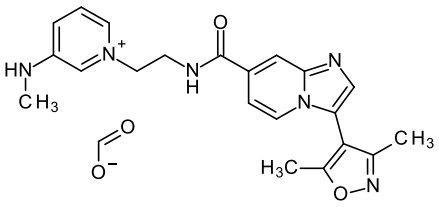
3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid:



In an argon atmosphere methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (2 g, 6.62 mmol) and tetrakis (triphenylphosphine) palladium (0) (383 mg, 0.33 mmol) were dissolved in 53 ml of DME. (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (2.33 g, 16.55 mmol), potassium carbonate (1.83 g, 13.24 mmol) and 26 ml of water were added and the mixture was stirred at 75 °C for 48 h. The reaction mixture was acidified with 1 N HCl and the solvents were removed on a rotary evaporator. The residue was suspended in dichloromethane / methanol 1:1, the insoluble solid was filtered off and the filtrate was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 5 % MeOH -> 40 % MeOH; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 790 mg (100 % purity, 46 % yield) of the title compound were obtained.

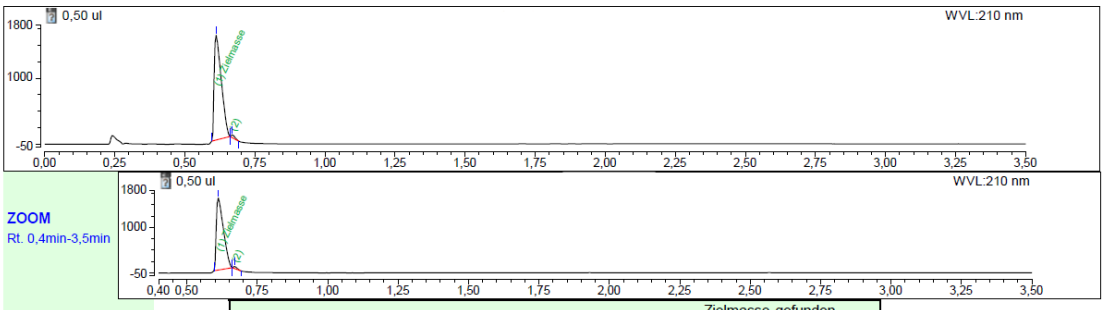
LC-MS (Method 5): Rt = 0.31 min; MS (ESIpos): m/z = 258 $[M+H]^+$

1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-3-(methylamino)pyridinium formate (30):



1-(2-Azaniumylethyl)-3-(methylamino)pyridinium dibromide (73 mg, 75 % purity, 0.17 mmol), DMAP (85 mg, 0.70 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (67 mg, 0.35 mmol) were added to a solution of 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid (60 mg, 0.23 mmol) in 3 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 43 mg (99 % purity, 42 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.61 min; MS (ESIpos): m/z = 391 $[M-HCOO]^+$

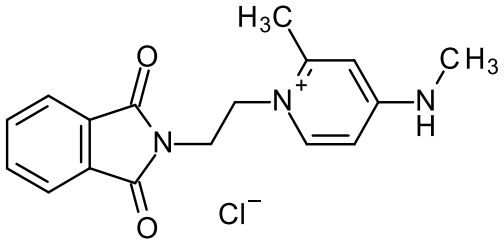


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,61	98,5	99,0	391,1877	
2	Y	0,67	1,5	1,0		

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.88 (m, 1H), 8.38 (m, 1H), 8.25 (d, J = 7.09 Hz, 1H), 8.17-8.11 (m, 3H), 7.87 (s, 1H), 7.70 (m, 1H), 7.59 (m, 1H), 7.26 (d, J = 7.21 Hz, 1H), 7.18 (m, 1H), 4.62 (br. s, 2H), 3.86 (m, 2H), 2,74 (s, 3H), 2.32 (s, 3H), 2.11 (s, 3H).

Preparation of (31) and respective starting materials:

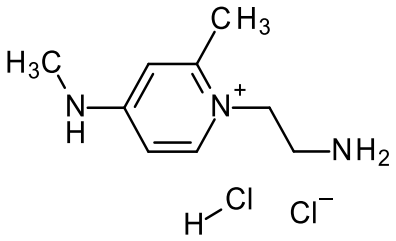
1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2-methyl-4-(methylamino)pyridinium chloride:



N,2-Dimethylpyridin-4-amine (895 mg, 7.32 mmol) was dissolved in 15 ml of DMF. 2-(2-chloroethyl)-1H-isoindole-1,3(2H)-dione (1.54 g, 7.32 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with ethyl acetate and dried in vacuo. The crude product was purified by flash chromatography on silica gel (eluent: DCM/MeOH/HCOOH 100/10/1 -> 100/30/1). Product containing samples were united and the solvents were evaporated. 312 mg (100 % purity, 13 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.45 min; MS (ESIpos): m/z = 296 $[M-Cl]^+$

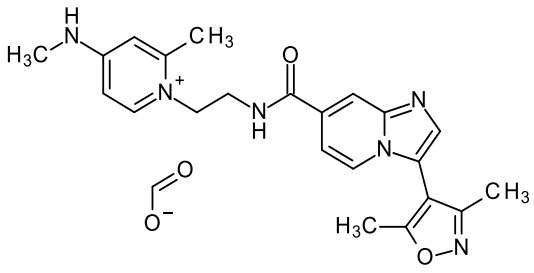
1-(2-aminoethyl)-2-methyl-4-(methylamino)pyridinium chloride hydrochloride:



A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2-methyl-4-(methylamino)pyridinium chloride (310 mg, 0.93 mmol) in 1.5 ml of conc. HCl was stirred at a bath temperature of 100 °C overnight. The reaction solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction through a frit and washed with water. The filtrate was concentrated at a rotary evaporator. The residue was stirred with THF/ACN/MeOH. The solid was filtered off and dried in vacuo. 189 mg (90 % purity, 74 % yield) of the title compound were obtained.

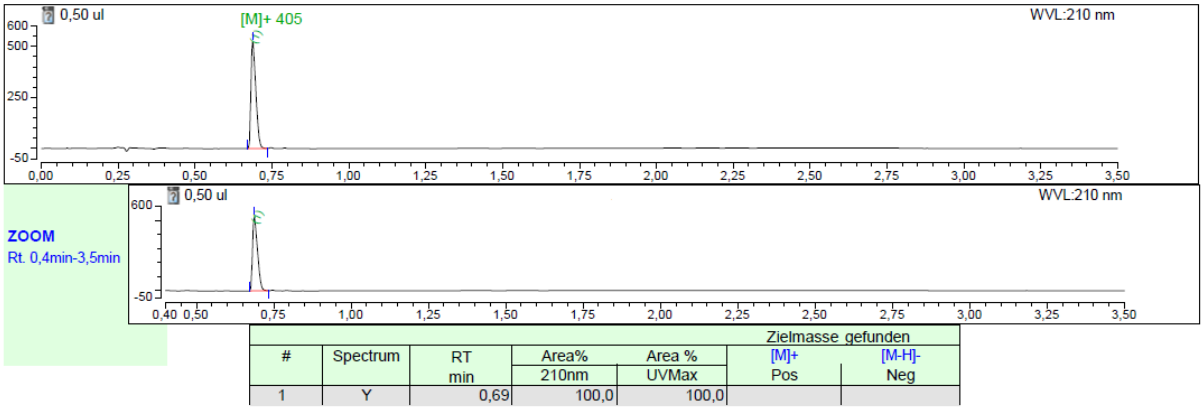
LC-MS (Method 4): Rt = 0.82 min; MS (ESIpos): m/z = 166 $[M-2Cl-H]^+$

1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-2-methyl-4-(methylamino)pyridinium formate (31):

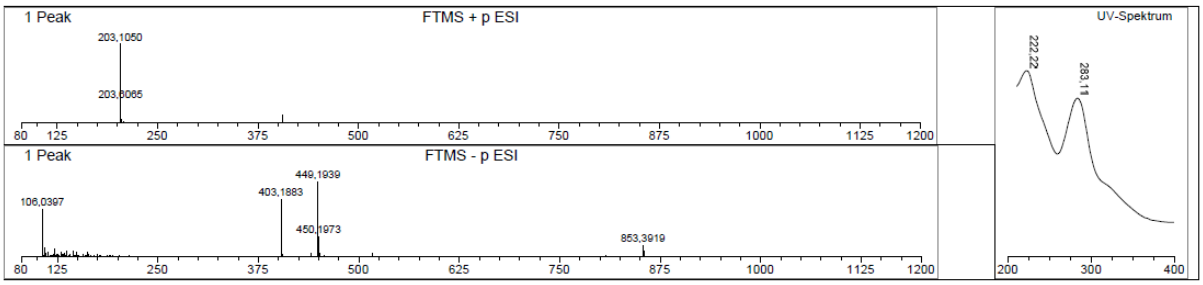


1-(2-Aminoethyl)-2-methyl-4-(methylamino)pyridinium chloride hydrochloride (47 mg, 90 % purity, 0.18 mmol), DMAP (66 mg, 0.54 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (51 mg, 0.27 mmol) were added to a solution of sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (50 mg, 0.19 mmol) in 2 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 36 mg (100 % purity, 45 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.69 min; MS (ESIpos): m/z = 405 $[M\text{-}HCOO]^+$



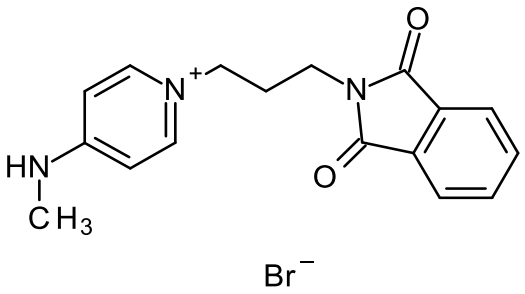
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,69	100,0	100,0		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.46-9.25 (m, 1H), 9.01-8.78 (m, 1H), 8.54 (br s, 1H), 8.24 (d, J = 6.48 Hz, 1H), 8.17 (s, 1H), 8.03 (m, 1H), 7.85 (s, 1H), 7.29 (d, J = 6.72 Hz, 1H), 6.81-6.68 (m, 2H), 4.34 (m, 2H), 3.71 (m, 2H), 2.85 (m, 3H), 2.54 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H).

Preparation of (32) and respective starting materials:

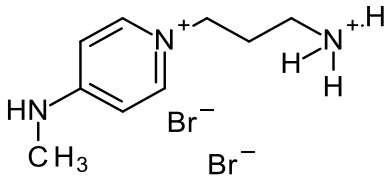
1-[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]-4-(methylamino)pyridinium bromide:



N-Methylpyridin-4-amine (2 g, 18.49 mmol) was dissolved in 20 ml of DMF. 2-(3-bromopropyl)-1H-isoindole-1,3(2H)-dione (4.96 g, 18.49 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE and dried in vacuo. 5.62 g (100 % purity, 81 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.83 min; MS (ESIpos): m/z = 296 $[M-Br]^+$

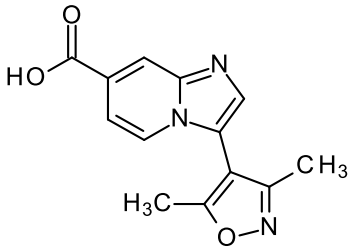
1-(3-aminopropyl)-4-(methylamino)pyridinium dibromide:



A solution of 1-[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]-4-(methylamino)pyridinium bromide (5.62 g, 14.94 mmol) in 21 ml of HBr (48 % in water) was stirred at a bath temperature of 100 °C overnight. The solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction. The filtrate was concentrated at a rotary evaporator and the residue was stirred with THF. The solid was filtered off, washed THF and dried in vacuo. 3.75 g (100 % purity, 77 % yield) of the title compound were obtained.

LC-MS (Method 4): Rt = 1.41 min; MS (ESIpos): m/z = 166 $[M-2Br]^+$

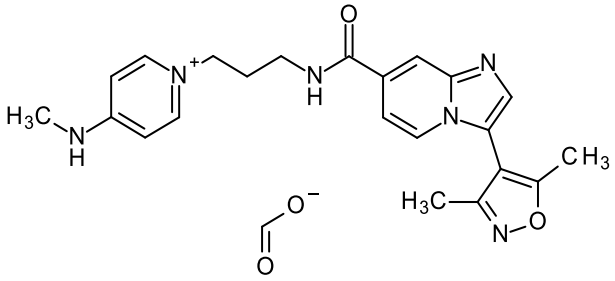
3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid:



In an argon atmosphere methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (2 g, 6.62 mmol) and tetrakis (triphenylphosphine) palladium (0) (383 mg, 0.33 mmol) were dissolved in 53 ml of DME. (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (2.33 g, 16.55 mmol), potassium carbonate (1.83 g, 13.24 mmol) and 26 ml of water were added and the mixture was stirred at 75 °C for 48 h. The reaction mixture was acidified with 1 N HCl and the solvents were removed on a rotary evaporator. The residue was suspended in dichloromethane / methanol 1:1, the insoluble solid was filtered off and the filtrate was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 5 % MeOH -> 40 % MeOH; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 790 mg (100 % purity, 46 % yield) of the title compound were obtained.

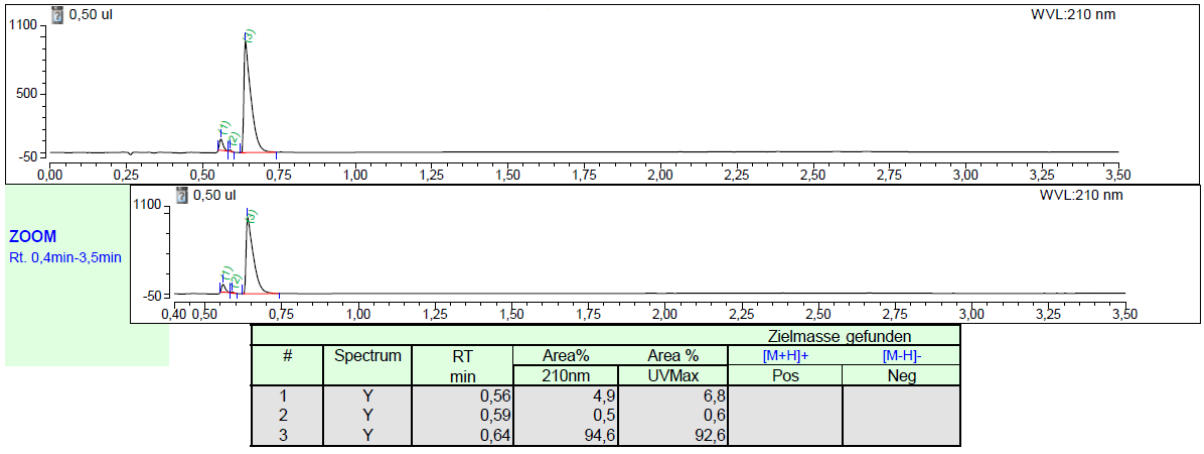
LC-MS (Method 5): Rt = 0.31 min; MS (ESIpos): m/z = 258 $[M+H]^+$

1-[3-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)propyl]-4-(methylamino)pyridinium formate (32):

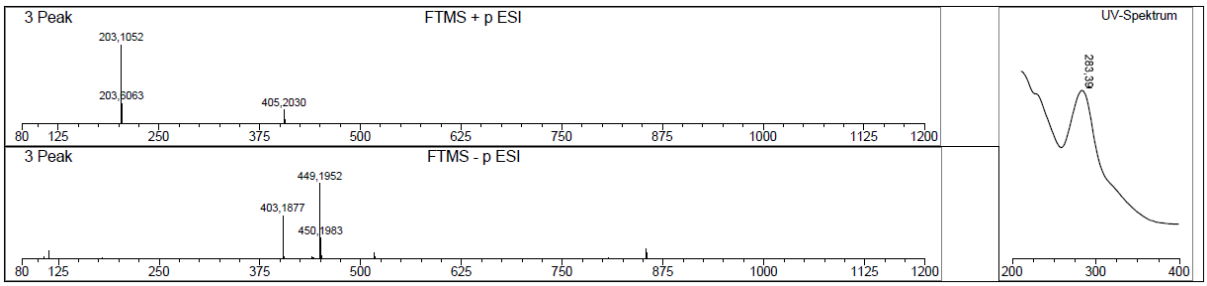


1-(3-Aminopropyl)-4-(methylamino)pyridinium dibromide (64 mg, 0.19 mmol), DMAP (71 mg, 0.58 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (56 mg, 0.29 mmol) were added to a solution of 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylic acid (50 mg, 0.19 mmol) in 5 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid) Product containing samples were united and the solvents were evaporated. 41 mg (95 % purity, 44 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.64 min; MS (ESIpos): m/z = 405 $[M-HCOO]^+$



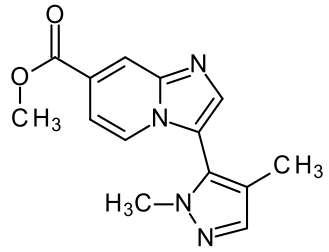
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	Zielmasse gefunden [M-H]- Neg
1	Y	0,56	4,9	6,8		
2	Y	0,59	0,5	0,6		
3	Y	0,64	94,6	92,6		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.16-8.86 (m, 2H), 8.44 (s, 1H), 8.37 (d, J = 7.34 Hz, 1H), 8.23 (m, 2H), 8.16 (d, J = 7.27 Hz, 1H), 7.87 (s, 1H), 7.37 (d, J = 8.19 Hz, 1H), 6.92 (d, J = 6.85 Hz, 1H), 6.86 (d, J = 7.21 Hz, 1H), 4.22 (t, J = 6.85 Hz, 2H), 3.32 (q, J = 6.24 Hz, 2H), 2.86 (d, J = 4.65 Hz, 3H), 2.33 (s, 3H), 2.12 (s, 3H), 2.06 (m, 2H).

Preparation of (33) and respective starting materials:

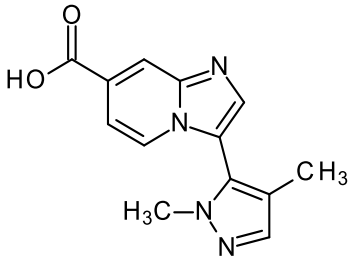
methyl 3-(1,4-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate:



Methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (250 mg, 0.83 mmol), 1,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (221 mg, 0.99 mmol) and potassium carbonate (377 mg, 2.73 mmol) were placed in 5 ml of dioxane and the mixture was degassed with argon for 10 minutes. Then [1,1-bis (diphenylphosphino) ferrocene] dichloropalladium dichloromethane complex (33.8 mg, 0.04 mmol) was added and the mixture was stirred at 110 °C overnight. The reaction mixture was concentrated. The residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 2 % MeOH -> 20 % MeOH; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 122 mg (74 % purity, 40 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.61 min; MS (ESIpos): m/z = 271 $[M+H]^+$

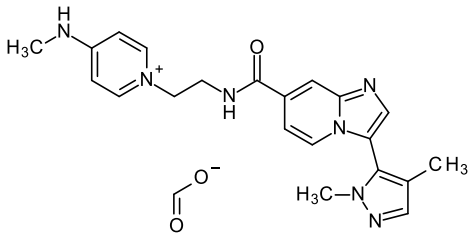
3-(1,4-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylic acid:



Methyl 3-(1,4-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate (122 mg, 74 % purity, 0.33 mmol) was dissolved in 7 ml of THF/water 3:1. Lithium hydroxide (22 mg, 0.9 mmol) was added and the mixture was stirred at room temperature for 2 h. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Reprosil C18 10 µm, 250 x 30 mm, detection: 210 nm, eluent A = water, B = acetonitrile; gradient: 10 % ACN (5.0 min) -> 80 % ACN (25 min) -> 95 % ACN (25.50 min –> 30.00 min) -> 10 % ACN (30.05 min –> 35.00 min); flow: 50 ml/min). Product containing samples were united and the solvents were evaporated. 59 mg (100 % purity, 70 % yield) of the title compound were obtained.

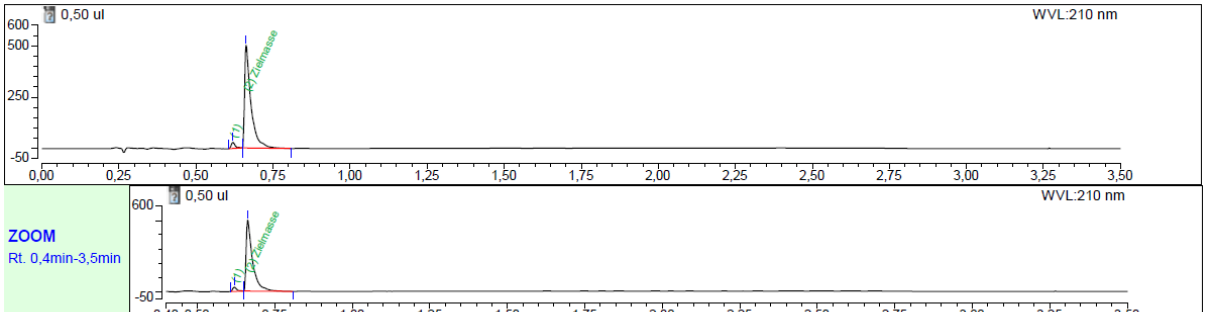
LC-MS (Method 2): Rt = 0.62 min; MS (ESIpos): m/z = 256 $[M+H]^+$

1-[2-({[3-(1,4-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium formate (33):



1-(2-Azaniumylethyl)-4-(methylamino)pyridinium dibromide (72 mg, 0.23 mmol), DMAP (84 mg, 0.69 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (66 mg, 0.35 mmol) were added to a solution of 3-(1,4-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylic acid (59 mg, 0.23 mmol) in 5 ml of dichloromethane and the mixture was stirred at room temperature for 1 h. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 41 mg (96 % purity, 39 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.66 min; MS (ESIpos): m/z = 390 $[M\text{-}HCOO]^+$

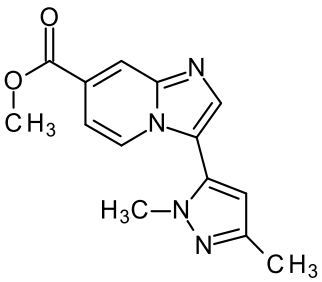


#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M]+ Pos	[M-H]- Neg
1	Y	0,62	3,7	4,3		
2	Y	0,66	96,3	95,7	390,2037	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.21-9.10 (m, 1H), 9.05-8.91 (m, 1H), 8.56 (s, 1H), 8.32 (d, J = 7.34 Hz, 1H), 8.20 (s, 1H), 8.12 (d, J = 6.85 Hz, 1H), 8.05 (d, J = 7.21 Hz, 1H), 7.98 (s, 1H), 7.51 (s, 1H), 7.33 (d, J = 7.21 Hz, 1H), 6.86 (m, 2H), 4.33 (t, J = 5.07 Hz, 2H), 3.72 (m, 2H), 3.65 (s, 3H), 2.86 (s, 3H), 1.87 (s, 3H).

<u>Preparation of (34) and respective starting materials:</u>

methyl 3-(1,3-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate:

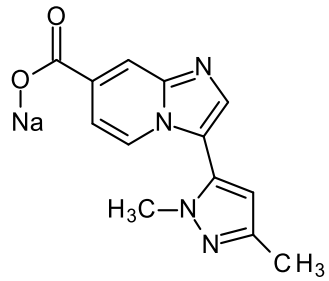


Methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (300 mg, 0.99 mmol), 1,3-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (441 mg, 1.99 mmol) and caesium fluoride (453 mg,

2.98 mmol) were placed in 6 ml of DMF and the mixture was degassed with argon for 10 minutes. Then [1,1-bis (diphenylphosphino) ferrocene] dichloropalladium dichloromethane complex (41 mg, 0.05 mmol) was added and the mixture was stirred 48 h at 90 °C. The reaction mixture was taken up in ethyl acetate and saturated sodium hydrogen carbonate solution. The mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 150 g; eluent: Cy / EE: 12 % EE -> 100 % EE -> DCM / MeOH 1.1; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 76 mg (100 % purity, 28 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.17 min; MS (ESIpos): m/z = 271 $[M+H]^+$

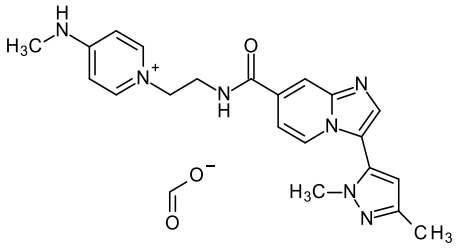
sodium 3-(1,3-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate:



Methyl 3-(1,3-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate (76 mg, 0.28 mmol) was dissolved in 1.6 ml of THF/water 3:1. Sodium hydroxide solution (56 mg, 1 M, 0.56 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with 4 N HCl and concentrated. The residue was stirred with acetonitrile, the solid was filtered off and dried in vacuo. 94 mg (100 % purity) of the title compound were obtained.

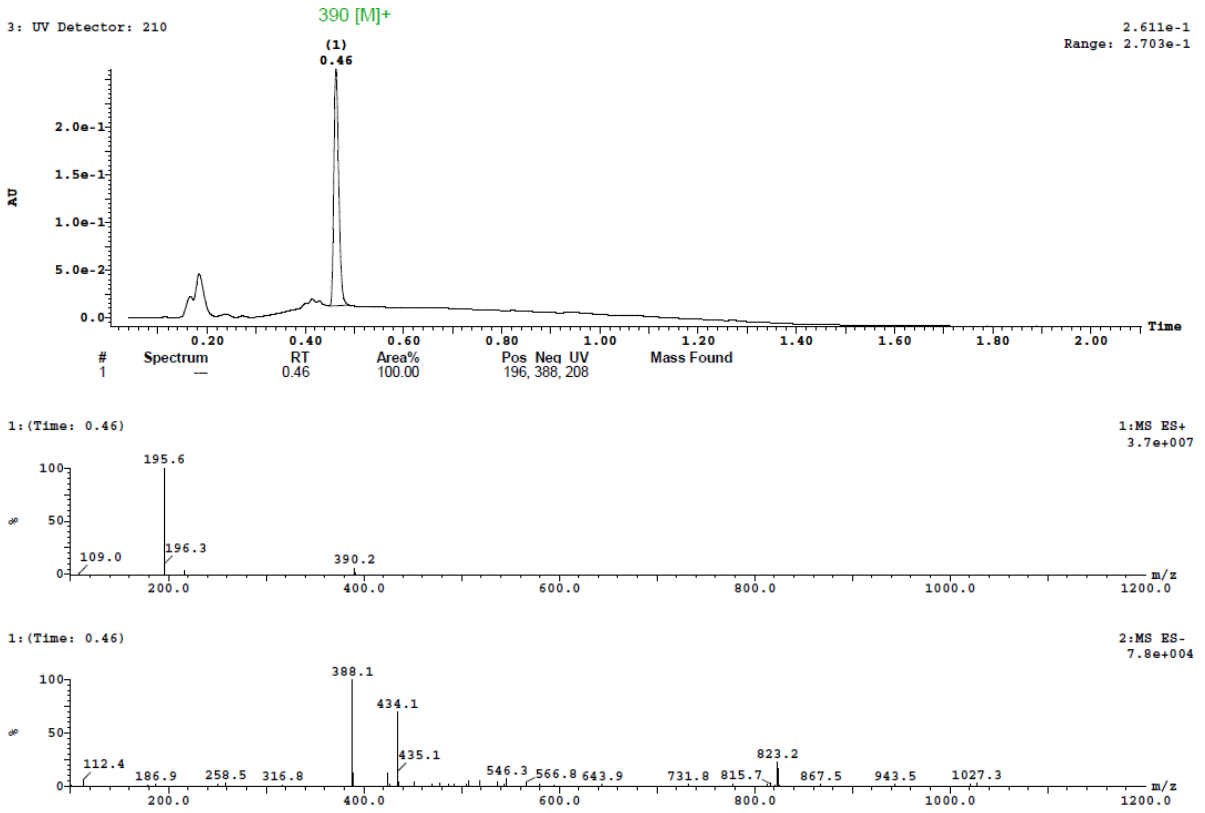
LC-MS (Method 2): Rt = 0.62 min; MS (ESIpos): m/z = 257 $[M-Na+2H]^+$

1-[2-({[3-(1,3-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium formate (34):



1-(2-Azaniumylethyl)-4-(methylamino)pyridinium dibromide (94 mg, 0.34 mmol), DMAP (124 mg, 1.01 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (97 mg, 0.51 mmol) were added to a solution of sodium 3-(1,3-dimethyl-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine-7-carboxylate (94 mg) in 17 ml of dichloromethane and the mixture was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 4 mg (100 % purity, 3 % yield) of the title compound were obtained.

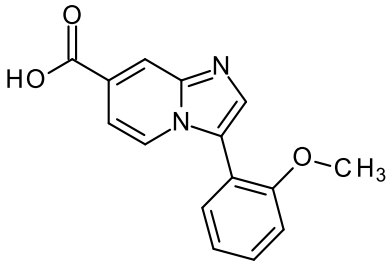
LC-MS (Method 1): Rt = 0.46 min; MS (ESIpos): m/z = 390 $[M-HCOO]^+$



^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.29 (t, J = 5.4 Hz, 1H), 9.13 (s, 1H), 8.57 (s, 1H), 8.37 (d, J = 7.09 Hz, 1H), 8.32 (dd, J = 7.41 Hz, 1H), 8.2 (s, 1H), 8.13 (dd, J = 7.33 Hz, 1H), 8.02 (s, 1H), 7.35 (dd, J = 7.21 Hz, 1H), 6.89 (dd, J = 7.29 Hz, 1H), 6.83 (dd, J = 7.41 Hz, 1H), 6.51 (s, 1H), 4.30 (t, J = 5.24 Hz, 2H), 3.76 (s, 3H), 3.72 (q, J = 4.97 Hz, 2H), 2.86 (s, 3H), 2.25 (s, 3H).

Preparation of (35) and respective starting materials:

3-(2-methoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylic acid:

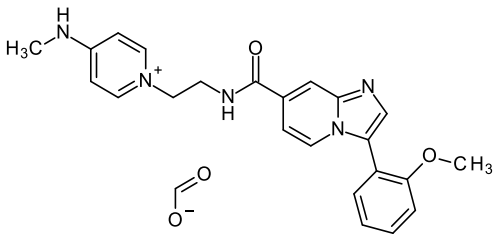


In an argon atmosphere methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (1 g, 3.31 mmol) and tetrakis (triphenylphosphine) palladium (0) (191 mg, 0.17 mmol) were dissolved in 27 ml of DME. (2-methoxyphenyl)boronic acid (1.26 g, 8.28 mmol), potassium carbonate (915 mg, 6.62 mmol) and 6.7 ml of water were added and the mixture was stirred at 75 °C overnight. Sodium hydroxide solution (3.31 ml, 1 M, 3.31 mmol) was added and the mixture was stirred at 75 °C for 48 h. The solid was filtered off and washed with DME / water. The combined filtrates were acidified with HCl (pH4) and the DME was removed on a rotary evaporator. The aqueous residue was diluted with brine and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP Ultra 50 g; eluent: DCM / MeOH: 5 % MeOH -> 10 % MeOH + 0.45 % formic acid; flow: 100

ml/min). Product containing samples were united and the solvents were evaporated. 785 mg (100 % purity, 84 % yield) of the title compound were obtained.

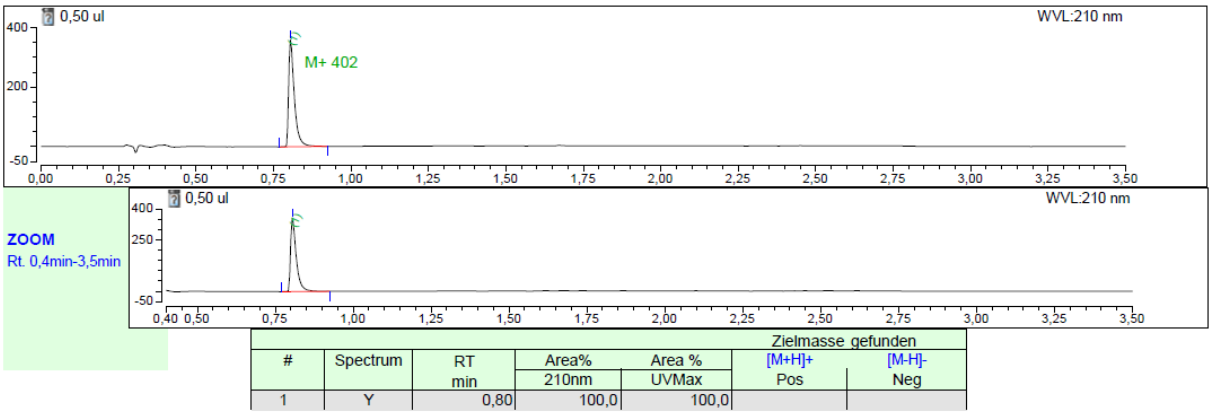
LC-MS (Method 2): Rt = 0.85 min; MS (ESIpos): m/z = 269 $[M+H]^+$

1-[2-({[3-(2-methoxyphenyl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium formate (35):

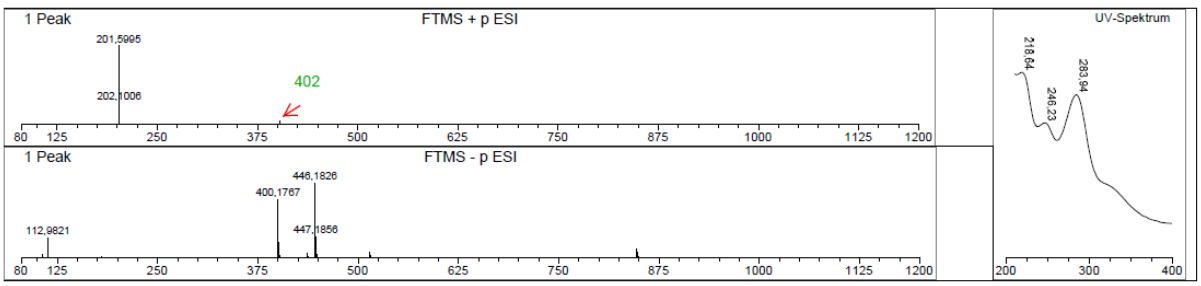


3-(2-Methoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylic acid (100 mg, 0.37 mmol) was dissolved in 2 ml of dichloromethane. 1-chloro-N,N,2-trimethylprop-1-en-1-amine (0.1 ml, 0.75 mmol) was added and the mixture was stirred at room temperature for 30 min. After that time pyridine (0.26 ml, 1.49 mmol) and 1-(2-azaniumylethyl)-4-(methylamino)pyridinium dibromide (140 mg, 0.45 mmol), dissolved in a mixture of 1 ml of dichloromethane and triethylamine (0.16 ml, 1.12 mmol) were added and the reaction was stirred at room temperature overnight. The solvent was removed at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B; 28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 54 mg (100 % purity, 32 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.80 min; MS (ESIpos): m/z = 402 $[M-HCOO]^+$



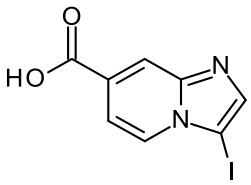
#	Spectrum	RT min	Area% 210nm	Area % UVMax	Zielmasse gefunden [M+H]+ Pos	[M-H]- Neg
1	Y	0,80	100,0	100,0		



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.07-8.82 (m, 2H), 8.40 (s, 1H), 8.31 (d, J = 6.97 Hz, 1H), 8.15-8.09 (m, 2H), 7.99 (d, J = 7.21 Hz, 1H), 7.78 (s, 1H), 7.54 (t, J = 7.95 Hz, 1H), 7.44 (dd, J = 7.52 Hz, 1H), 7.28-7.22 (m, 2H), 7.13 (t, J = 7.46 Hz, 1H), 6.86 (m, 2H), 4.33 (t, J = 5.2 Hz, 2H), 3.79 (s, 3H), 3.72 (q, J = 5.34 Hz, 2H), 2.86 (d, J = 4.65 Hz, 3H).

Preparation of (36) and respective starting materials:

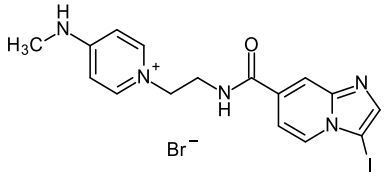
3-iodoimidazo[1,2-a]pyridine-7-carboxylic acid:



Methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (2 g, 6.62 mmol) was dissolved in 40 ml of THF. An aqueous lithium hydroxide solution (13.2 ml, 1 M, 13.2 mmol) was added and the mixture was stirred at room temperature for 1 h. The organic solvent was removed at a rotary evaporator and the aqueous residue was acidified with 4 N HCl. The precipitated solid was filtered off, washed with acetonitrile and dried in vacuo. 1.66 g (100 % purity, 87 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.68 min; MS (ESIpos): m/z = 288 $[M+H]^+$

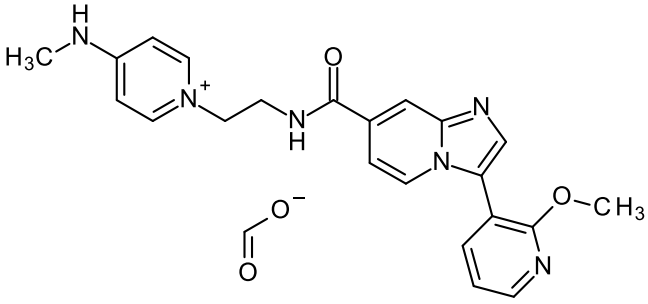
1-(2-{[(3-iodoimidazo[1,2-a]pyridin-7-yl)carbonyl]amino}ethyl)-4-(methylamino)pyridinium bromide:



1-(2-Azaniumylethyl)-4-(methylamino)pyridinium dibromide (924 mg, 2.95 mmol), DMAP (1.08 g, 8.85 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (849 mg, 4.43 mmol) were added to a solution of 3-iodoimidazo[1,2-a]pyridine-7-carboxylic acid (850 mg, 2.95 mmol) in 17 ml of dichloromethane and the mixture was stirred at room temperature for 2h. The precipitated solid was filtered off, washed with dichloromethane and dried in vacuo. 1.2 g (100 % purity, 81 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.72 min; MS (ESIpos): m/z = 422 $[M-Br]^+$

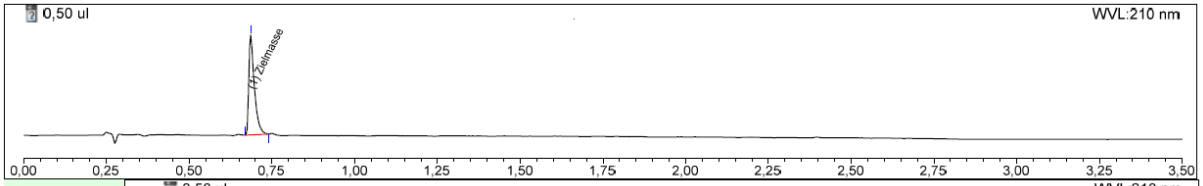
1-[2-({[3-(2-methoxypyridin-3-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium formate (36):



1-(2-{[(3-Iodoimidazo[1,2-a]pyridin-7-yl)carbonyl]amino}ethyl)-4-(methylamino)pyridinium bromide (300 mg, 0.60 mmol), (2-methoxypyridin-3-yl)boronic acid (183 mg, 1.20 mmol) and potassium carbonate (248 mg, 1.79 mmol) were treated with 7.5 ml of dioxane/water 4:1 and the mixture was degassed with argon for 10 minutes. Then [1,1-bis (diphenylphosphino) ferrocene] dichloropalladium dichloromethane complex (44 mg, 0.06 mmol) was added and the mixture was stirred at 90 °C for 3 h. The reaction mixture was diluted with methanol and filtered through celite. The filtrate was concentrated at a rotary evaporator and the crude product was purified by preparative HPLC (Column: Chromatorex C18 10 µm 250 x 30 mm; Eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 10 min 5 % B; 23 min 95 % B;

28 min 95 % B; 29 min 10 % B; flow: 75 ml/min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 141 mg (100 % purity, 53 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.69 min; MS (ESIpos): m/z = 403 [M-HCOO]$^+$

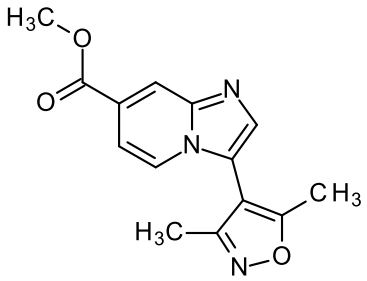


			Zielmasse gefunden			
#	Spectrum	RT	Area%	Area %	[M]+	[M-H]-
		min	210nm	UVMax	Pos	Neg
1	Y	0,69	100,0	100,0	403,1877	

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.10 (t, J = 5.67 Hz, 1H), 8.97 (q, J = 4.41 Hz, 1H), 8.51 (s, 1H), 8.35 (dd, J = 5.04 Hz, 1H), 8.31 (dd, J = 7.33 Hz, 1H), 8.17-8.10 (m, 3H), 7.92 (dd, J = 7.33 Hz, 1H), 7.86 (s, 1H), 7.28 (dd, J = 7.21 Hz, 1H), 7.19 (dd, J = 7.29 Hz, 1H), 6.90-6.81 (m, 2H), 4.33 (m, 2H), 3.90 (s, 3H), 3.72 (q, J = 5.6 Hz, 2 H), 2.86 (d, J = 4.73 Hz, 3H).

Preparation of (39):

Methyl 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (39):



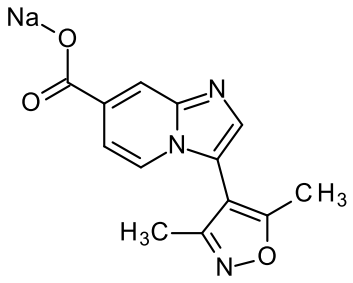
Methyl 3-iodoimidazo[1,2-a]pyridine-7-carboxylate (10 g, 33.11 mmol) was dissolved in 500 ml of DMF, (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid (9.33 g, 66.21 mmol) and cesium fluoride (15.09 g, 99.31 mmol) were added to the solution and the mixture was degassed with argon for 10 min. Then [1,1-bis (diphenylphosphino)ferrocene]dichloropalladium (II) (1.35g, 1.66 mmol) was added and the mixture was stirred at 90 °C overnight. The precipitated solid was filtered off and discarded. The filtrate was concentrated to half, diluted with water and saturated $NaHCO_3$-solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, and evaporated. The crude product was purified by column chromatography (Machine: Biotage® Isolera; column: Biotage® SNAP KP-Sil 100 g Ultra; eluent: Cy / EE: 12 % EE ->100 % EE; flow: 100 ml/min). Product containing samples were united and the solvents were evaporated. 4.85 g (100 % purity, 54 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 1.09 min; MS (ESIpos): m/z = 272 [M+H]$^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.28 (m, 1H), 8.24 (s, 1H), 7.95 (s, 1H), 7.35 (dd, J = 7.09 Hz, 1H), 3.91 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H).

Preparation of (40):

Sodium 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (40):



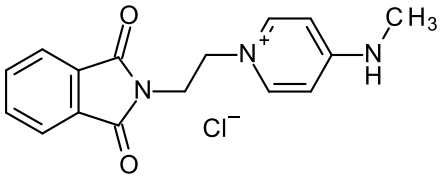
Methyl 3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridine-7-carboxylate (3.94 g, 14.52 mmol) was dissolved in 80 ml of THF / MeOH 3:1. Sodium hydroxide solution (29.05 ml, 1M, 29.05 mmol) was added and the mixture was stirred at room temperature. After 30 min the reaction mixture was neutralized with 4N HCl and concentrated. The precipitate was stirred with methanol and insoluble solids were filtered off and discarded. The filtrate was concentrated, and the residue was stirred with acetonitrile. The solid was filtered off and dried in vacuo. 3.87 g (100 % purity, 95 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.54 min; MS (ESIpos): m/z = 258 $[M-Na+2H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 8.51 (br d, J = 6.36 Hz, 1H), 8.39 (s, 1H), 8.34 (s, 1H), 7.62 (br d, J = 6.97 Hz, 1H), 2.38 (s, 3H), 2.16 (s, 3H).

Preparation of (42):

1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium chloride (42):



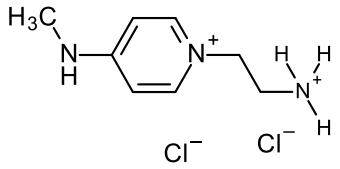
N-Methylpyridin-4-amine (15.5 g, 143.11 mmol) was dissolved in 125 ml of DMF. 2-(2-chloroethyl)-1H-isoindole-1,3(2H)-dione (30.0 g, 143.11 mmol) was added to the solution and the mixture was stirred at 110 °C overnight. The precipitated solid was filtered off, washed with MTBE, and dried in vacuo. 28.6 g (100 % purity, 63 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.67 min; MS (ESIpos): m/z = 282 $[M-Cl]^+$

^{1}H-NMR (500 MHz, DMSO-d_6) δ [ppm] = 9.05 (m, 1H), 8.35 (dd, J = 7.41 Hz, 1H), 8.15 (dd, J = 7.33 Hz, 1H), 7.88-7.84 (m, 4H), 6.88 (dd, J = 7.29 Hz, 1H), 6.79 (dd, J = 7.41 Hz, 1H), 4.32 (m, 2H), 3.94 (m, 2H), 2.84 (d, J = 4.97 Hz, 3H).

Preparation of (43):

1-(2-azaniumylethyl)-4-(methylamino)pyridinium dichloride (43):



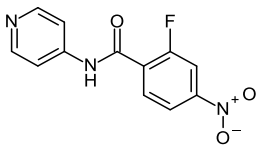
A solution of 1-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(methylamino)pyridinium chloride (22.9 g, 72.06 mmol) in 120 ml of conc. HCl was stirred at a bath temperature of 100 °C over the weekend. The reaction solution was cooled to 0 °C, the phthalic acid which had precipitated was filtered off with suction through a frit (por. 3) and washed with water (2 × 20 ml). The filtrate was concentrated at a bath temperature of 70 °C on a rotary evaporator and then coevaporated twice with 250 ml of dichloromethane. The light brown residue was stirred overnight with 250 ml of THF. The solid was filtered off, washed two times with 50 ml of diisopropyl ether and dried in vacuo. 15.3 g (100 % purity, 95 % yield) of the title compound were obtained.

LC-MS (Method 1): Rt = 0.14 min; MS (ESIpos): m/z = 152 $[M-2Cl]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.13 (m, 1H), 8.63 (m, 3H), 8.39 (d, J = 7.21 Hz, 1H), 8.21 (d, J = 6.72 Hz, 1H), 7.00-6.91 (m, 2H), 4.42 (t, J = 5.75 Hz, 2H), 3.27 (m, 2H), 2.90 (d, J = 4.89 Hz, 3H).

Preparation of (44) and respective starting materials:

2-fluoro-4-nitro-N-(pyridin-4-yl)benzamide:

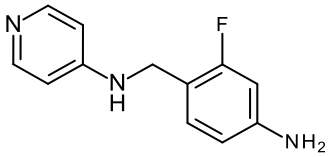


2-Fluoro-4-nitrobenzoic acid (20g, 108.04 mmol) and pyridin-4-amine (11.19 g, 118.85 mmol) were suspended in 500 ml of dichloromethane. The suspension was cooled to 0 °C. EDC*HCl (24.86 g, 129.65 mmol) and DMAP (132 mg, 1.08 mmol) were added, and the mixture was stirred at room temperature overnight. Water was added and the precipitated solid was filtered off, washed with water and dried in vacuo. 20.5 g (99 % purity, 72 % yield) were obtained.

LC-MS (Method 2): Rt = 0.72 min; MS (ESIpos): m/z = 262 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 11.1 (s, 1H), 8.52 (m, 2H), 8.31 (dd, J = 9.66 Hz, 1H), 8.21 (dd, J = 8.44 Hz, 1H), 7.99 (m, 1H), 7.68 (m, 2H).

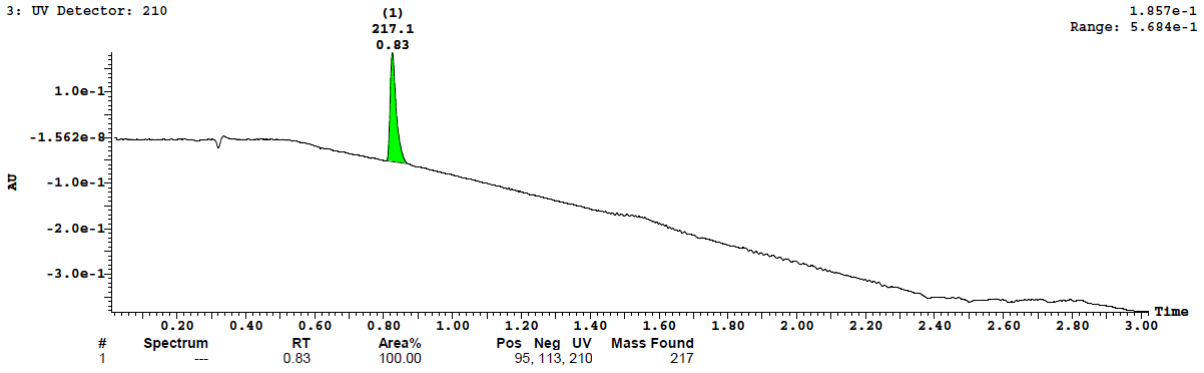
N-(4-amino-2-fluorobenzyl)pyridin-4-amine (45):



Under an argon atmosphere 2-fluoro-4-nitro-N-(pyridin-4-yl)benzamide (20.5 g, 99 % purity, 77.7 mmol) was dissolved in 400 ml of THF. Borane-THF-complex (194.24 ml, 1 M, 194.24 mmol) was added and the mixture was refluxed overnight. The mixture was diluted with 200 ml of 1 N HCl and stirred for 10 min. Then 1 l of water was added, and the mixture was extracted with ethyl acetate. The combined

organic layers were dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: EE/MeOH 10:1 -> 0:1). Product containing samples were united and the solvents were evaporated. 14.97 g of a mixture of N-(2-fluoro-4-nitrobenzyl)pyridin-4-amine and N-(4-amino-2-fluorobenzyl)pyridin-4-amine was isolated. This mixture was dissolved in 450 ml of methanol. Palladium (10% on activated carbon, 3.22 g, 3.03 mmol) and ammonium formate (38.18 g, 605.51 mmol) were added, and the mixture was refluxed for 3 h. The catalyst was filtered off through Celite, the filtrate was concentrated, and the residue was extracted between ethyl acetate and water. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (eluent: EE/MeOH 1:0 -> 10:1). Product containing samples were united and the solvents were evaporated. 5.71 g (100 % purity, 43.4 % yield) were obtained.

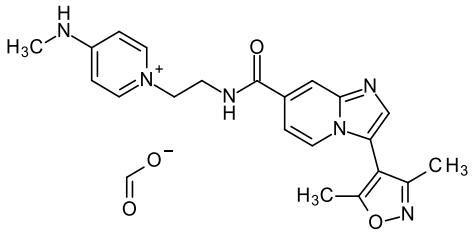
LC-MS (Method 4): Rt = 0.83 min; MS (ESIpos): m/z = 218 $[M+H]^+$



^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 7.98 (d, J = 5.36 Hz, 2H), 6.99 (t, J = 8.59 Hz, 1H), 6.78 (br. s, 1H), 6.48 (dd, J = 5.28 Hz, 2H), 6.34 (s, 1H), 6.30 (m, 1H), 5.32 (s, 2H), 4.10 (d, J = 5.36 Hz, 2H).

Preparation of (45):

1-[2-({[3-(3,5-dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino)pyridinium formate (44):



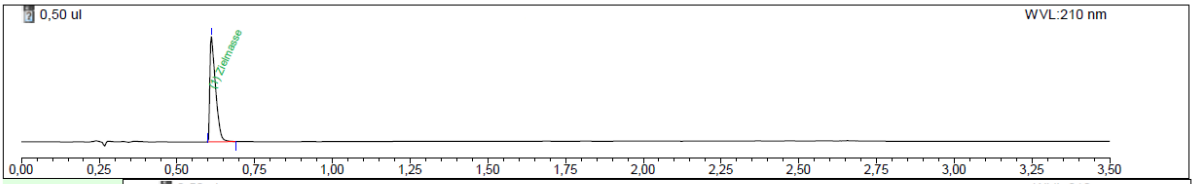
Step 1: Loading the ion exchange resin:

90 ml of Amberlite IRA 410 chloride form were filled into an empty cartridge. 500 ml of a 1 M aqueous sodium formate solution were passed through the resin, followed by 500 ml of water.

Step 2: Exchange chloride / formate:

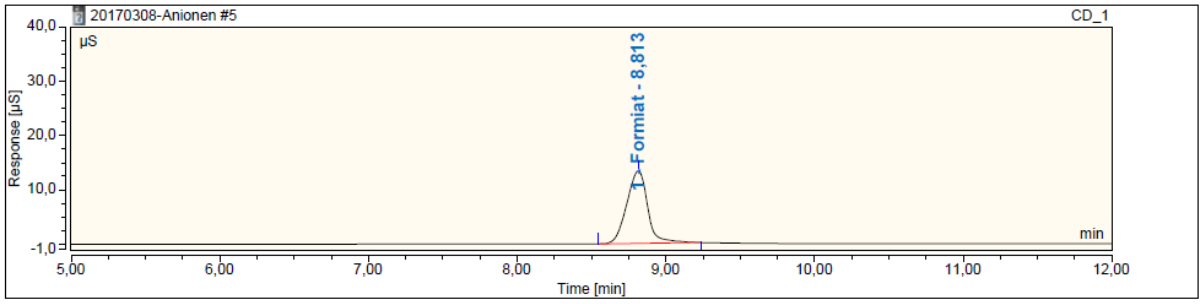
1-[2-({[3-(3,5-Dimethyl-1,2-oxazol-4-yl)imidazo[1,2-a]pyridin-7-yl]carbonyl}amino)ethyl]-4-(methylamino) pyridinium chloride (24) (1.00 g, 2.34 mmol) was dissolved in 3 ml of water and passed through the ion exchange resin described in step 1. The resin was washed with 250 ml of water, and the combined filtrates were concentrated and dried in vacuo. The residue was purified by preparative HPLC (column: Chromatorex C18 10 µm, 250 × 30 mm, eluent A = water, B = acetonitrile; gradient: 0.0 min 5 % B; 3 min 5 % B; 20 min 50 % B; 23 min 100 % B; 26 min 5 % B; flow: 50 ml / min; 0.1 % formic acid). Product containing samples were united and the solvents were evaporated. 886 mg (100 % purity, 87 % yield) of the title compound were obtained.

LC-MS (Method 2): Rt = 0.34 min; MS (ESIpos): m/z = 391 [M-HCOO]$^+$



					Zielmasse gefunden	
#	Spectrum	RT min	Area% 210nm	Area % UVMax	[M]+ POS	[M-H]- Neg
1	Y	0,61	100,0	100,0	391,18765	

^{1}H-NMR (400 MHz, DMSO-d_6) δ [ppm] = 9.23-9.12 (m, 2H), 8.48 (s, 1H), 8.31 (dd, J = 7.4 1H), 8.23 (d, J = 7.21 Hz, 1H), 8.18 (s, 1H), 8.12 (d, J = 7.21 Hz, 1H), 7.86 (s, 1H), 7.29 (dd, J = 7.15 Hz, 1H), 6.89 (d, J 7.21 Hz, 1H), 6.83 (d, J = 6.85 Hz, 1H), 4.34 (br. t, J = 5.26 Hz, 2H), 3.71 (q, J = 5.5 Hz, 2H), 2.86 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H).



No.	Name	Time min	Massenanteil in (Gew. %) Formiat
2	Leer	n.a.	n.a.
5		8,81	11,7
6		8,55	11,6
	Average:	8,68	11,6

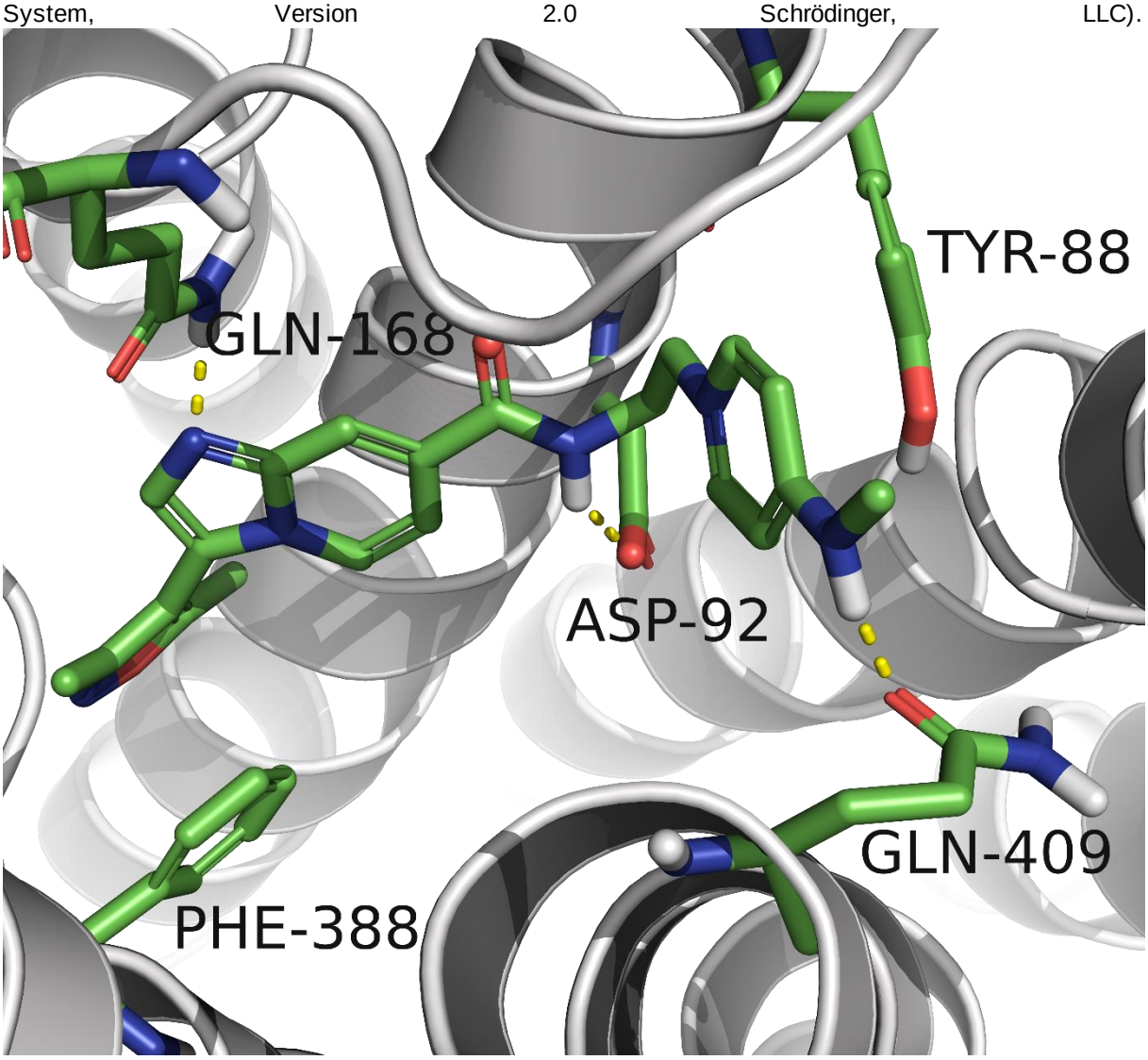
Ion chromatography: w(formate) = 11.6 weight % = 1.1 eq. (formate).

Homology Model

A homology model of the α_{2B} receptor was build based on an x-ray structure of the β2-receptor using Schrödinger's Prime Software (3nya.pdb, http://doi.org/10.2210/pdb3NYA/pdb, http://dx.doi.org/10.1021/ja105108q, Schrödinger Release 2016-2: Prime, Schrödinger, LLC, New York, NY, 2021.). Schrödinger's Induced Fit Docking was applied to find a suitable binding pose for compound **12** (Schrödinger Release 2016-2: Induced Fit Docking protocol; Glide, Schrödinger, LLC, New York, NY,

2021; Prime, Schrödinger, LLC, New York, NY, 2021). To gain insight into binding of ligands similar to compound **12** complexes of these compounds and the receptor were constructed mimicking the found binding pose of compound **12**. After ligand placement the complexes were optimized. (Schrödinger Release 2016-2, MacroModel, Schrödinger, LLC, New York, NY, 2021.). Compound **24** was accommodated with small shifts in the side chains of the protein adjusting for the larger size of the amide portion of the antagonist.

Figure S5. Binding pose of compound **24** in a homology model of the α_{2B} receptor. GLN-168, ASP-92 and PHE-388 are directly interacting with the ligand. Crucial hydrogen bond and charge interactions are shown as dotted yellow lines. The figure has been prepared with Pymol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).



Abbreviations

Abbreviation	Meaning
Ac	acetate
ACN	acetonitrile
AcOH	acetic acid

AG	Aktiengesellschaft
aq.	aqueous
AR	Adrenergic receptor
br	broad (^{1}H-NMR signal)
Caco	colorectal adenocarcinoma cells
CHO	chinese hamster ovary
CI	chemical ionisation
CL	clearance
conc.	concentrated
Cy	cyclohexane
CYP	cytochrome P enzyme
d	days (duration of reaction)
d	doublet (^{1}H-NMR signal)
DCM	dichloromethane
dd	double-doublet (^{1}H-NMR signal)
ddd	doublet of doublets of doublets
DIPEA	N,N-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DME	Dimethoxyethane
DMF	N,N-Dimethylformamide
DMSO	dimethylsulfoxide
EDC*HCl	N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride
EE	ethyl acetate
ent	enantiomerically pure compound
eq.	equivalents
ESI	electrospray (ES) ionisation
Et	ethyl
EtOH	ethanol
Et_2O	diethyl ether
h	hour(s) (duration of reaction)
h	human (e.g. cell line)
$hCa_v1.2$	human L-type Ca channel
hERG	human Ether-a-go-go channel
$hK_{ir}2.1$	human potassium inward rectifying channel

$hNa_v1.5$	human voltage-gated sodium channel subunit
HPLC	high performance liquid chromatography
HTS	high throughput screening
IC_{50}	concentration leading to 50% inhibition
iv	intravenous
kg	kilogram
L	litre(s)
LC-MS	liquid chromatography mass spectrometry
NaHMDS	sodium bis(trimethylsilyl)amide
M	mass (in mass spectrometry)
M	molar (concentration)
m	multiplet
Me	CH_3
MeOH	methanol
mg	milligram
MHz	megahertz
µM	micromolar
min	minute(s)
ml	millilitre(s)
MRT	mean residence time
MS	mass spectrometry
MTBE	2-methoxy-2-methylpropane
m/z	mass/charge
N	normal (concentration)
nM	nanomolar
NMR	nuclear magnetic resonance spectroscopy: chemical shifts (δ) are given in ppm. The chemical shifts were corrected by setting the DMSO signal to 2.50 ppm unless otherwise stated.
NMP	N-methylpyrrolidinone
PEG	polyethylene glycol
PK	pharmacokinetic
ppm	parts per million
q	quartet
rac	racemic mixture
RP	Reversed-Phase chromatography

Rt	retention time (as measured either with HPLC or UPLC) in minutes
r.t.	room temperature
s	singlet (^{1}H-NMR signal)
sat.	saturated
SQD	Single-Quadrupole-Detector
t	triplet (^{1}H-NMR signal)
td	triple-doublet (^{1}H-NMR signal)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane
uHTS	ultra high throughput screening
UV	ultraviolet
Vss	volume of distribution