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DATASHEET

LUF7909

Product overview

Name	LUF7909
Cat No	HB4786
Biological description	Novel, adenosine A_1AR Affinity-Based Probe (AfBP) which is suitable for click conjugation for use in confocal microscopy, SDS-PAGE and chemical proteomics profiling applications. Labeling of the A_1AR is more specific in live CHOhA1AR cells compared to labeling in membrane fractions. LUF7909 acts as a partial agonist which is highly specific to the A_1AR and binds covalently (apparent pK_i values at A_1AR are 7.8 and 9.5 (following a 4h preincubation), where a K_i shift indicates a covalent mode of action).

Applications

Live cells or membrane fractions should be incubated with LUF7909 to selectively label the desired receptor in the presence of other proteins.

The desired reporter group can subsequently be clicked onto the probe, effectively labeling the receptor.

Finally, the reporter-bound receptor is processed based on the detection method (e.g. confocal microscopy, SDS-PAGE, chemical proteomics)

Please see our protocol booklet: LUF7909 (HB4786) Protocol

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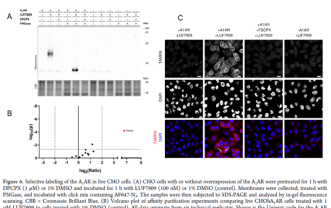
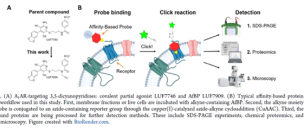
Biological action	Agonist
Purity	>95%
Description	Novel, clickable Adenosine hA_1AR Affinity-Based Probe (AfBP).

Images





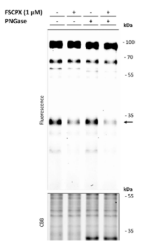
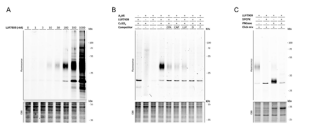


Figure 7. Labeling of the A_1AR in adipocyte membranes derived from mouse gonadal fat pads. The membranes were pretreated with the covalent antagonist FSCPX (1 μM) or 1% DMSO prior to incubation with LUF7909 (100 nM) and subsequent incubation with click mix containing AF647-N_3. The samples were then denatured, subjected to SDS-PAGE, and analyzed using in-gel fluorescence scanning. CBB = Coomassie Brilliant Blue. The band that appears upon Coomassie staining (lanes 3 and 4) corresponds to the molecular weight of PNGase.



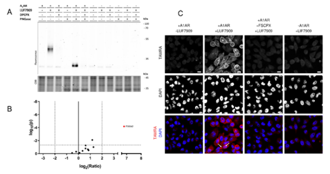


Figure 6. Selective labeling of the A_1AR in live CHO cells. (A) CHO cells with or without overexpression of the A_1AR were pretreated for 1 h with DPCPX (1 μM) or 1% DMSO and incubated for 1 h with LUF7909 (100 nM) or 1% DMSO (control). Membranes were collected, treated with PNGase, and incubated with click mix containing AF647-N_3. The samples were then subjected to SDS-PAGE and analyzed by in-gel fluorescence scanning. CBB = Coomassie Brilliant Blue. (B) Volcano plot of affinity purification experiments comparing live CHOhA_1AR cells treated with 1 μM LUF7909 to cells treated with 1% DMSO (control). All data originate from six technical replicates. Shown is the Uniprot code for the A_1AR (P30542) (highlighted in red). (C) Confocal microscopy images. CHO cells with or without overexpression of the A_1AR were pretreated for 1 h with FSCPX (1 μM) or 1% DMSO and incubated for 1 h with LUF7909 (100 nM) or 1% DMSO (control). The cells were then fixed and stained with TAMRA-N_3 (first row) and DAPI (second row). The third row shows an overlay of both stains. TAMRA = red, DAPI = blue. Arrows indicate examples of labeled membranes and labeling inside cells. Images were selected manually as representatives of blinded measurements from two separate experiments (see Figure S8). Scale bar = 10 μm. Figure was created using OMERO.[52]

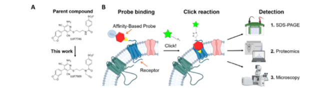


Figure 1. (A) A_1AR-targeting 3,5-dicyanopyridines: covalent partial agonist LUF7746 and AfBP LUF7909. (B) Typical affinity-based protein profiling workflow used in this study. First, membrane fractions or live cells are incubated with alkyne-containing AfBP. Second, the alkyne moiety of the probe is conjugated to an azide-containing reporter group through the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). Third, the probe-bound proteins are being processed for further detection methods. These include SDS-PAGE experiments, chemical proteomics, and confocal microscopy. Figure created with BioRender.com.

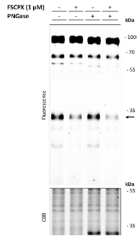


Figure 7. Labeling of the A_1AR in adipocyte membranes derived from mouse gonadal fat pads. The membranes were pretreated with the covalent antagonist FSCPX (1 μM) or 1% DMSO prior to incubation with LUF7909 (100 nM) and subsequent incubation with click mix containing AF647-N_3. The samples were then denatured, subjected to SDS-PAGE, and analyzed using in-gel fluorescence scanning. CBB = Coomassie Brilliant Blue. The band that appears upon Coomassie staining (lanes 3 and 4) corresponds to the molecular weight of PNGase.

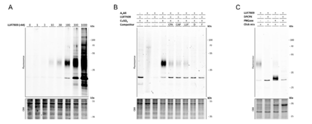


Figure 4. Specific labeling of the A_1AR using LUF7909 in CHO membranes overexpressing the A_1AR. Membranes were preincubated with or without competitor, incubated with LUF7909, subsequently "clicked" to AF647-N_3, denatured, subjected to SDS-PAGE, and analyzed using in-gel fluorescence scanning. (A) Concentration-dependent labeling of the A_1AR. (B) Labeling of the A_1AR is dependent on the presence of copper(I) and probe during the click reaction, as well as the presence of known A_1AR ligands: agonist CPA, partial agonist CAP, covalent partial agonist LUF7746 (LUF), antagonist DPCPX (D), and covalent antagonist FSCPX (F; structures in Figure S3). (C) Labeling of the A_1AR shows a strong reduction in molecular weight upon incubation with PNGase. Preincubation with 1 μM DPCPX shows full disappearance of both bands. CBB = Coomassie Brilliant Blue. The band that appears upon Coomassie staining (lanes 3 and 4) corresponds to the molecular weight of PNGase.

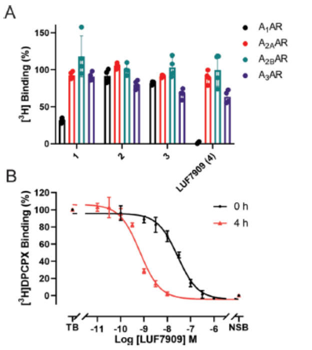


Figure 2. Affinities of LUF7909 and analogues for the four adenosine receptor subtypes. (A) Displacement of [^{3}H]DPCPX (A_1AR), [^{3}H]ZM241385 ($A_{2A}AR$), [^{3}H]PSB-603 ($A_{2B}AR$), and [^{3}H]PSB-11 (A_3AR) binding by 1 μM of the respective AfBP. Data represent the values of two individual experiments performed in duplicate and are normalized to the vehicle control (100%). (B) Displacement of [^{3}H]DPCPX from the A_1AR by LUF7909 measured after 0 or 4 h of preincubation of LUF7909 with CHO membranes stably overexpressing the A_1AR. TB = total radioligand binding (vehicle control); NSB = nonspecific radioligand binding. Data represent the mean ± SEM of three individual experiments performed in duplicate.

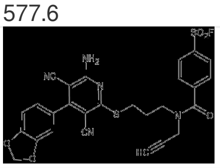
Biological Data

Application notes	**Please see our protocol booklet: LUF7909 (HB4786) Protocol**

Solubility & Handling

Storage instructions	-20°C
Solubility overview	Soluble in DMSO
Storage of solutions	Prepare and use solutions on the same day if possible. Store solutions at -20°C for up to one month if storage is required. Equilibrate to RT and ensure the solution is precipitate free before use.
Shipping Conditions	Stable for ambient temperature shipping. Follow storage instructions on receipt.
Important	This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Chemical Data

Chemical name	4-((3-((6-Amino-4-(benzo[d][1,3]dioxol-5-yl)-3,5-dicyanopyridin-2-yl)thio)propyl)(prop-2-yn-1-yl)carbamoyl)benzenesulfonyl fluoride
Molecular Weight	577.6
Chemical structure	
Molecular Formula	$C_{27}H_{20}FN_5O_5S_2$
PubChem identifier	167312224
SMILES	NC1=C(C#N)C(C2=CC=C3OCOC3=C2)=C(C#N)C(SCCCN(CC#C)C(=O)C2=CC=C(C=C2)S(F)(=O)=O)=N1
InChiKey	DGFSACSPMOOCNR-UHFFFAOYSA-N
Licensing details	Sold under license from the Oncode Cancer Institute and Universiteit Leiden

References

A Chemical Biological Approach to Study G Protein-Coupled Receptors: Labeling the Adenosine A(1) Receptor Using an Electrophilic Covalent Probe.

Beerkens BLH et al (2022) ACS chemical biology 17

PubMedID 36279267