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Letter

Small Molecule Fluorescent Ligands for the Atypical Chemokine Receptor 3 (ACKR3)

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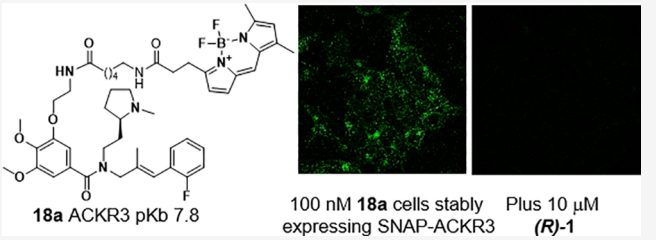
ABSTRACT: The atypical chemokine receptor 3 (ACKR3) is a receptor that induces cancer progression and metastasis in multiple cell types. Therefore, new chemical tools are required to study the role of ACKR3 in cancer and other diseases. In this study, fluorescent probes, based on a series of small molecule ACKR3 agonists, were synthesized. Three fluorescent probes, which showed specific binding to ACKR3 through a luminescence-based NanoBRET binding assay (pK_d ranging from 6.8 to 7.8) are disclosed. Due to their high affinity at the ACKR3, we have shown their application in both competition binding experiments and confocal microscopy studies showing the cellular distribution of this receptor.



KEYWORDS: *Chemokine receptor, ACKR3, CXCR7, Fluorescent probes, BODIPY, NanoBRET*

The atypical chemokine receptor 3 (ACKR3), previously known as CXC-chemokine receptor 7 (CXCR7), is an atypical chemokine receptor belonging to the class A G protein-coupled receptor (GPCR) family. Although the

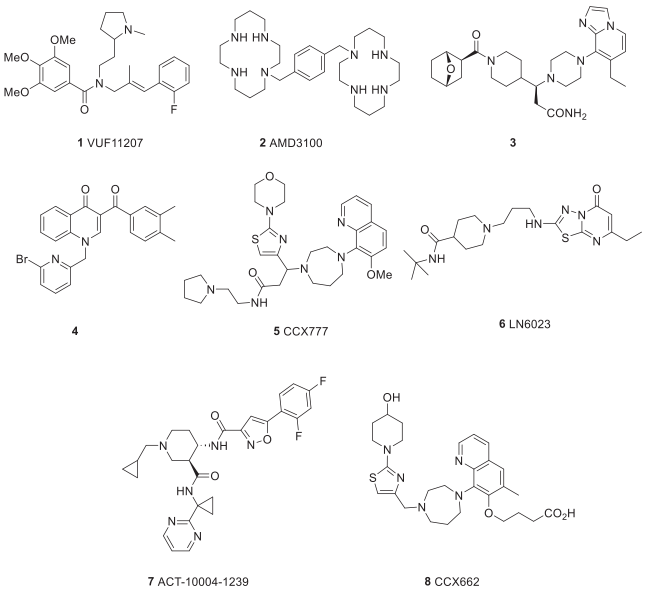


Figure 1. Examples of reported small molecule ACKR3 ligands: **1**,[10] **2**,[11] **3**,[12] **4**,[13] **5**,[14] **6**,[15] **7**,[16] and **8**.[17] Details of the affinity or potency of the compounds are shown in the Supporting Information (Table S1).

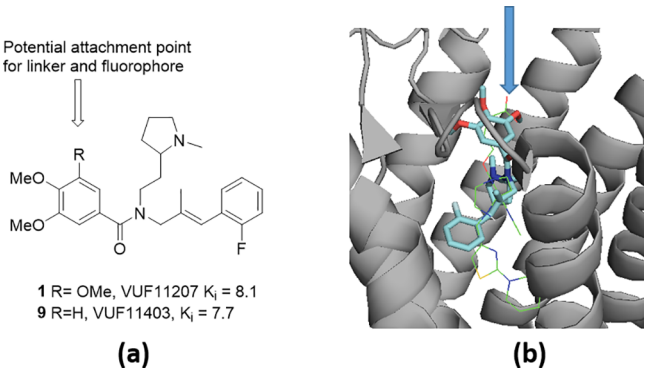


Figure 2. (a) Reported SAR[10] suggests the highlighted 3-methoxy group present in VUF11207 (**1**) is not essential for ACKR3 binding and can be targeted for linker and fluorophore attachment (b) Docking of VUF11207 (*R*)-**1** into ACKR3 (pdb 7SK9) suggests substitution on the 3-position of the aryl ring would be an appropriate choice for linker and fluorophore attachment. Docking experiments were performed using OEDOCKING Hybrid docking.[24,25]

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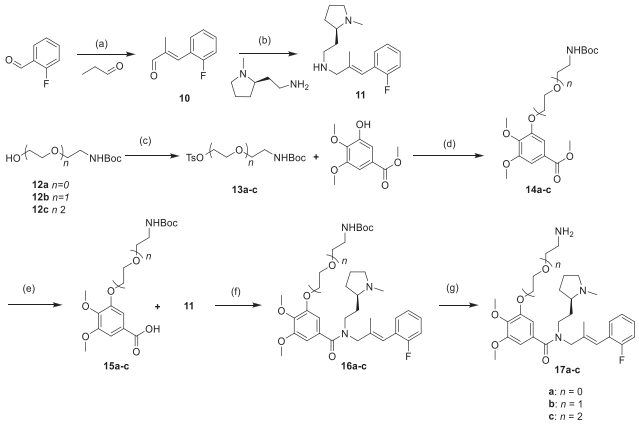
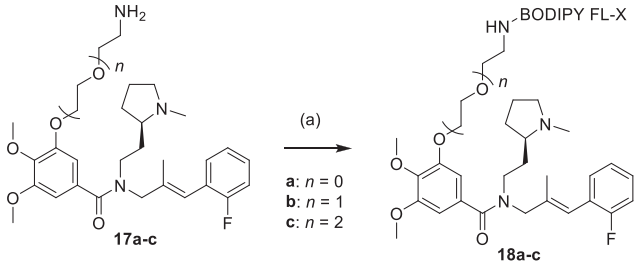
Scheme 1. Synthesis of Fluorescent Ligand Precursor's 17a−c[a]



[a]Reagents and conditions: (a) KOH, ethanol, water, stir rt 24 h, 90%; (b) picoline borane complex, methanol, acetic acid, rt, 24 h, 63%; (c) triethylamine, tosyl chloride, DCM, rt, 24 h, 36−40%; (d) cesium carbonate, DMF, rt, 24 h, 63−83%; (e) lithium hydroxide, THF, water, rt, 24 h, quant; (f) HATU, Hunig's base, DMF, rt, 72−77%; (g) TFA, DCM, rt, quant.

Scheme 2. Synthesis of Fluorescent ACKR3 Ligands 18a−c[a]



[a]Reagents and conditions: (a) BODIPY FL-X succinimidyl ester, Hunig's base, acetonitrile, 24 h, extrusion of light (56−77%).

biological role of ACKR3 is not entirely understood, it is reported to function as a scavenger of CXCL12 (C-X-C chemokine 12, also known as SDF-1, stromal cell-derived factor 1) establishing CXCL12 gradients, thereby modulating CXCR4 signaling.[1,2] It has been postulated to regulate a range of biological functions that occur after binding of the endogenous ligand CXCL12 and subsequent recruitment of the multifunctional intracellular protein β-arrestin, resulting in phosphorylation-dependent receptor internalization without detectable activation of G-proteins.[3]

Expression of ACKR3 on the surface of platelets has been shown to be up-regulated in patients suffering with acute myocardial infarction and subsequent elevation of ACKR3 expression leads to an improvement in recovery.[4,5] Additionally, increased infarct size and subsequent patient mortality have been observed, where ACKR3 expression has been decreased, signifying the importance of ACKR3 in promoting proliferation and angiogenesis.[6] ACKR3 is known to be overexpressed in numerous cancer types, indicating its involvement in the modulation of tumor cell proliferation and migration and tumor angiogenesis, contributing to cancer progression and metastasis.[7] Due to the increasing literature for the role of ACKR3 in disease, several structurally diverse small molecule ACKR3 ligands have been reported (Figure 1).[8,9]

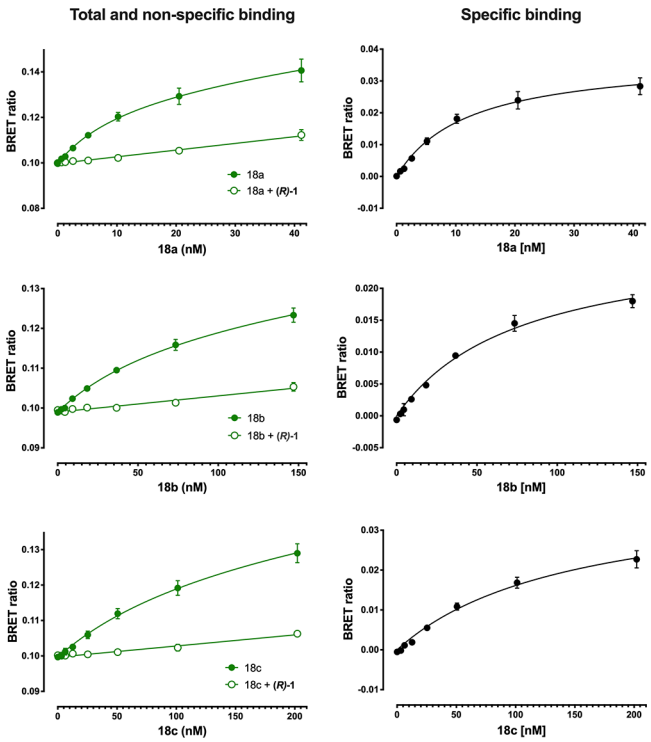


Figure 3. Saturation binding of (*R*)-**1** using **18a−c** in HEK293G_NLuc-ACKR3 cells. HEK293G_NLuc-ACKR3 cells were treated with **18a−c** in the presence and absence of 10 μM unlabeled (*R*)-**1** full-length *N* terminal NanoLuciferase-ACKR3 stably expressing HEK293 cells. Compounds were added simultaneously and incubated for 60 min at 37 °C in HBSS containing 0.2% BSA. Furimazine (1:400 final dilution) was added and plates incubated for 5 min. Fluorescence and luminescence emissions were measured using a BMG Pherastar FS. The raw BRET ratio was calculated by dividing the fluorescent signal by the bioluminescent signal and specific binding was calculated by deducting nonspecific binding from the total binding values.

Table 1. Binding Affinities of 18a−c Determined in HEK293G Cells Expressing NLuc-ACKR3

example	pK_d (log *M*)[a]	*n*
18a	7.89 ± 0.01	4
18b	7.09 ± 0.01	4
18c	6.82 ± 0.01	4

[a]pK_d values were calculated from the negative logarithm of the equilibrium dissociation constant (K_d) determined from saturation-binding experiments using increasing concentrations of labeled ligand in the presence or absence of (*R*)-**1** (10 μM). Data are expressed as mean ± SEM, where each experiment was performed in triplicate.

Table 2. Binding Affinities of a Series of Known ACKR3 Ligands

example*	pK_i (log *M*)[a]	*n*
4	7.4 ± 0.13	5
9	6.6 ± 0.07	6
19	5.0 ± 0.10	5
20	6.2 ± 0.07	6

[a]Data are combined mean ± SEM, where each experiment was performed in triplicate.

Currently, the most widely used compound to study ACKR3 function is the endogenous ligand CXCL12. Although human CXCL12 and its radiolabeled and fluorescently labeled

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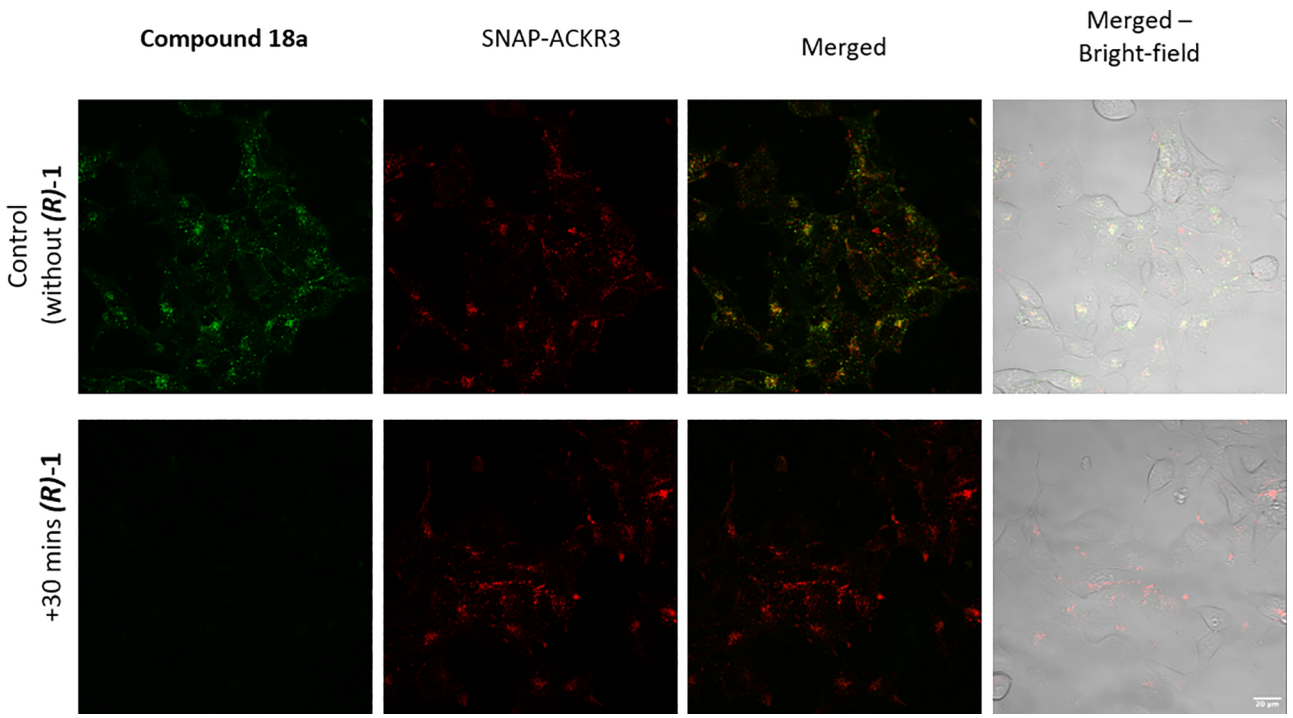

Figure 4. Binding of compound **18a** (100 nM). SNAP-ACKR3 receptor expression (red), Compound **18a** (green) overlay image (third column) with yellow indicating colocalization following incubation for 30 min at 37 °C. Bright-field images of the cells (4th column). Images were taken on a Zeiss LSM710 confocal microscope, 40× 1.2NA water-immersion objective, and representative of four independent experiments.

versions are available through commercial sources, their arduous synthesis makes them very expensive to employ in both *in vitro* and *in vivo* imaging. Antibodies and nanobodies have also emerged as highly selective tools to study ACKR3[18,19] but similar to CXCL12, the development of ACKR3-specific antibodies and nanobodies is difficult and time-consuming, making them also very expensive for the medicinal chemist to routinely employ.

Small molecule ligands that selectively target ACKR3 can offer several advantages over chemokines and antibodies as tool compounds to probe receptor function. Though their discovery may be challenging, they are generally more accessible and cheaper for synthetic chemists to make and fluorophore containing analogues offer the potential for detailed visualization of receptor function at a cellular level.[20−23]

We report the synthesis of the first fluorescent ACKR3 probes, based on the receptor agonist VUF11207 (**1**).[10] An evaluation of the reported structure−activity relationship (SAR) of the small molecule inhibitor, combined with *in silico* docking experiments utilizing the recently disclosed Cryo-EM structure of ACKR3 complexed with the partial agonist **8** CCX662,[17] informed the synthetic strategy for linker design and fluorophore attachment (Figure 2).

The resulting fluorescent compounds were characterized in a BRET-based assay, enabled by a NanoLuciferase (NLuc)-ACKR3 construct. The recently developed NanoBRET methodology has allowed characterization of various (fluorescent) probes targeting GPCRs, even when under endogenous promotion.[20,21,26]

The synthesis of fluorescent derivatives of VUF11207 was based on procedures that were described by Wijtmans in the development of VUF11207.[10] Zarca et al. recently reported on the pharmacological evaluation of the synthesized single enantiomers of VUF11207 (**1**) showing that (*R*)-**1** had a pEC_{50} of 8.3 ± 0.1 compared to (*S*)-**1**, which has a corresponding pEC_{50} of 7.7 ± 0.1 in a [^{125}I] CXCL12 displacement assay.[27]

Synthesis started with an aldol reaction between 2-fluorobenzaldehyde and propionaldehyde, which under basic conditions provided (*E*)-3-(2-fluorophenyl)-2-methylacrylaldehyde **10** in excellent yield. A reductive amination with a picoline borane complex and (*R*)-2-(1-methylpyrrolidin-2-yl)ethanamine gave the homochiral precursor **11** in good yield. With this key fragment in hand, we set out to synthesize the various linkers. Here, we chose to develop linkers of three different lengths, with PEG chains ranging from 0 to 2. Commercially available alcohol-carbamates **12a−c** were first converted into tosylates using tosyl chloride **13a−c**. *O*-Alkylation using methyl 3-hydroxy-4,5-dimethoxybenzoate efficiently installed the linkers on the 3′-position. Hydrolysis of the methyl ester to the benzoic acids **15a−c** using lithium hydroxide proceeded with quantitative yields, allowing subsequent peptide coupling with key intermediate **11** to give **16a−c** and after *N*-Boc deprotection, the congeners **17a−c** were ready for conjugation to commercially available fluorescent dyes (Scheme 1).

The congeners **17a−c** were reacted with the commercial BODIPY FL-X succinimidyl ester to give the corresponding fluorescent ligands **18a−c**, after purification by reverse phase HPLC. The fluorescent ligands were prepared in >95% purity as defined through analytical HPLC (Scheme 2).

Pharmacological Evaluation of Fluorescent ACKR3 Antagonists. The fluorescent conjugates (**18a−c**) were evaluated by using a range of pharmacological assays. Initially, saturation binding experiments were used to determine the affinity of the fluorescent conjugates toward the ACKR3 receptor. The fluorescent properties of the compounds allowed

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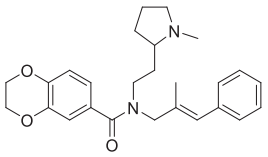
detection of the proximity of the fluorescent ligands to an *N*-terminal NanoLuciferase-tagged receptor (NLuc-ACKR3) by means of bioluminescence resonance energy transfer (NanoBRET).[20] The three fluorescent conjugates produced clear saturable specific binding to the NLuc-ACKR3 receptor that was associated with low levels of nonspecific binding (determined in the presence of unlabeled (*R*)-**1**) resulting in pK_d values ranging from 6.8 to 7.9 (Figure 3 and Table 1).

To further evaluate the use of **18a** in the NanoBRET-ligand binding assay, affinities of ACKR3 ligands **4**, **9**, **19**, and **20** were determined in competition binding experiments (Table 2).



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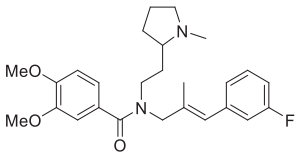

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The availability of high affinity green fluorescent ACKR3 receptor ligands suggested utility for live cell imaging. Confocal microscopy images of fluorescent ligand **18a** incubated with HEK293 cells transiently expressing *N*-terminal SNAPTag-ACKR3 (referred to as SNAP-ACKR3) for 30 min at 37 °C were captured. Under these conditions, SNAP-ACKR3 labeled with the cell impermeable SNAP-AF647 showed a predominantly vesicular intracellular location, with a small amount on the cell membrane (Figure 4, second column). This is consistent with its known high levels of constitutive ACKR3 cycling. Ligand **18a** (100 nM) showed a very similar distribution of mainly intracellular fluorescence, which was colocalized with that of the SNAP-ACKR3 receptor (Figure 4) and may also therefore indicate some ligand induced internalization. Images collected at various time points during incubation of 50 nM **18a** (Supporting Information, Figure S1) indicated that **18a** was initially bound to the cell surface at early time points and then internalized with SNAP-ACKR3. When cells were pretreated with (*R*)-**1**, its level of binding was significantly reduced, suggesting that the majority of observed fluorescence was specific binding of **18a** to the SNAP-ACKR3 receptor.

We have reported the characterization of the first new small molecule-based fluorescent probes for ACKR3. Compounds (**18a**−**c**) retained good affinity toward the ACKR3 receptor, as shown by NanoBRET saturation experiments. We further demonstrated that **18a** is a useful screening tool for discovering new ACKR3 agonists. Compound **18a** displayed good signal-to-noise in NanoBRET competition-binding experiments and was displaced by the established small molecule agonist (*R*)-**1**, close analogues, and a structurally diverse agonist **4**. The fluorescent ACKR3 ligands (**18a**−**c**) can be used in live cell confocal microscopy experiments and in combination with the NanoBRET approach may shed further light on ACKR3 function and its participation in pathophysiological conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00469.

Method for compound preparation, LCMS traces of **18a**−**c**, pharmacological methods, time course confocal imaging of **18a**, pharmacological data for selected literature compounds (PDF)

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Author Contributions

The manuscript was written and approved by all authors.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ACKR3, atypical chemokine receptor 3; BODIPY, boron dipyrromethene; BRET, bioluminescence resonance energy transfer; BSA, bovine serum albumin; CXCL12, C X-C chemokine ligand type 12; CXCR4, CXC-chemokine receptor type 4; CXCR7 CXC-chemokine receptor 7, DMF, dimethylformamide; HEK293G, human embryonic kidney cells expressing a GloSensor biosensor; LC/MS, liquid chromatography/mass spectrometry; NLuc, NanoLuciferase; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; RP-HPLC, reverse phase high performance liquid chromatography; SAR, structure−activity relationship; SDF, stromal cell-derived factor 1; TOF ES+, positive electrospray ionization time-of-flight

■ REFERENCES

(1) Levoye, A.; Balabanian, K.; Baleux, F.; Bachelerie, F.; Lagane, B. CXCR7 Heterodimerizes with CXCR4 and Regulates CXCL12-Mediated G Protein Signaling. *Blood* **2009**, *113* (24), 6085−6093.

(2) Naumann, U.; Cameroni, E.; Pruenster, M.; Mahabaleshwar, H.; Raz, E.; Zerwes, H.-G.; Rot, A.; Thelen, M. CXCR7 Functions as a Scavenger for CXCL12 and CXCL11. *PLoS One* **2010**, *5* (2), No. e9175.

(3) Rajagopal, S.; Kim, J.; Ahn, S.; Craig, S.; Lam, C. M.; Gerard, N. P.; Gerard, C.; Lefkowitz, R. J. *β*-Arrestin- but Not G Protein-Mediated Signaling by the "Decoy" Receptor CXCR7. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (2), 628−632.

(4) Rath, D.; Chatterjee, M.; Borst, O.; Muller, K.; Stellos, K.; Mack, A. F.; Bongartz, A.; Bigalke, B.; Langer, H.; Schwab, M.; Gawaz, M.; Geisler, T. Expression of Stromal Cell-Derived Factor-1 Receptors CXCR4 and CXCR7 on Circulating Platelets of Patients with Acute Coronary Syndrome and Association with Left Ventricular Functional Recovery. *Eur. Heart J.* **2014**, *35* (6), 386−394.

(5) Rath, D.; Chatterjee, M.; Meyer, L.; Tekath, N.; Olma, C.; Krumm, P.; Adams, C.; Borst, O.; Müller, K.; Droppa, M.; Nikolaou, K.; Riethmüller, J.; Gawaz, M.; Geisler, T. Relative Survival Potential of Platelets Is Associated with Platelet CXCR4/CXCR7 Surface Exposure and Functional Recovery Following STEMI. *Atherosclerosis* **2018**, *278*, 269−277.

(6) Hao, H.; Hu, S.; Chen, H.; Bu, D.; Zhu, L.; Xu, C.; Chu, F.; Huo, X.; Tang, Y.; Sun, X.; Ding, B.-S.; Liu, D.-P.; Hu, S.; Wang, M. Loss of Endothelial CXCR7 Impairs Vascular Homeostasis and Cardiac Remodeling After Myocardial Infarction. *Circulation* **2017**, *135* (13), 1253−1264.

(7) Neves, M.; Fumagalli, A.; van den Bor, J.; Marin, P.; Smit, M. J.; Mayor, F. The Role of ACKR3 in Breast, Lung, and Brain Cancer. *Mol. Pharmacol.* **2019**, *96* (6), 819−825.

(8) Lounsbury, N. Advances in CXCR7Modulators. *Pharmaceuticals* **2020**, *13* (2), 33.

(9) Adlere, I.; Caspar, B.; Arimont, M.; Dekkers, S.; Visser, K.; Stuijt, J.; de Graaf, C.; Stocks, M.; Kellam, B.; Briddon, S.; Wijtmans, M.; de Esch, I.; Hill, S.; Leurs, R. Modulators of CXCR4 and CXCR7/ACKR3 Function. *Mol. Pharmacol.* **2019**, *96*, 737.

(10) Wijtmans, M.; Maussang, D.; Sirci, F.; Scholten, D. J.; Canals, M.; Mujić-Delić, A.; Chong, M.; Chatalic, K. L. S.; Custers, H.; Janssen, E.; De Graaf, C.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. Synthesis, Modeling and Functional Activity of Substituted Styrene-Amides as Small-Molecule CXCR7 Agonists. *Eur. J. Med. Chem.* **2012**, *51*, 184−192.

(11) Kalatskaya, I.; Berchiche, Y. A.; Gravel, S.; Limberg, B. J.; Rosenbaum, J. S.; Heveker, N. AMD3100 Is a CXCR7 Ligand with Allosteric Agonist Properties. *Mol. Pharmacol.* **2009**, *75* (5), 1240−1247.

(12) Menhaji-Klotz, E.; Hesp, K. D.; Londregan, A. T.; Kalgutkar, A. S.; Piotrowski, D. W.; Boehm, M.; Song, K.; Ryder, T.; Beaumont, K.; Jones, R. M.; Atkinson, K.; Brown, J. A.; Litchfield, J.; Xiao, J.; Canterbury, D. P.; Burford, K.; Thuma, B. A.; Limberakis, C.; Jiao, W.; Bagley, S. W.; Agarwal, S.; Crowell, D.; Pazdziorko, S.; Ward, J.; Price, D. A.; Clerin, V. Discovery of a Novel Small-Molecule Modulator of C-X-C Chemokine Receptor Type 7 as a Treatment for Cardiac Fibrosis. *J. Med. Chem.* **2018**, *61* (8), 3685−3696.

(13) Uto-Konomi, A.; McKibben, B.; Wirtz, J.; Sato, Y.; Takano, A.; Nanki, T.; Suzuki, S. CXCR7 Agonists Inhibit the Function of CXCL12 by Down-Regulation of CXCR4. *Biochem. Biophys. Res. Commun.* **2013**, *431* (4), 772−776.

(14) Gustavsson, M.; Wang, L.; van Gils, N.; Stephens, B. S.; Zhang, P.; Schall, T. J.; Yang, S.; Abagyan, R.; Chance, M. R.; Kufareva, I.; Handel, T. M. Structural Basis of Ligand Interaction with Atypical Chemokine Receptor 3. *Nat. Commun.* **2017**, *8*, 14135.

(15) Bayrak, A.; Mohr, F.; Kolb, K.; Szpakowska, M.; Shevchenko, E.; Dicenta, V.; Rohlfing, A. K.; Kudolo, M.; Pantsar, T.; Günther, M.; Kaczor, A. A.; Poso, A.; Chevigné, A.; Pillaiyar, T.; Gawaz, M.; Laufer, S. A. Discovery and Development of First-in-Class ACKR3/CXCR7 Superagonists for Platelet Degranulation Modulation. *J. Med. Chem.* **2022**, *65* (19), 13365−13384.

(16) Richard-Bildstein, S.; Aissaoui, H.; Pothier, J.; Schäfer, G.; Gnerre, C.; Lindenberg, E.; Lehembre, F.; Pouzol, L.; Guerry, P. Discovery of the Potent, Selective, Orally Available CXCR7 Antagonist ACT-1004−1239. *J. Med. Chem.* **2020**, *63* (24), 15864−15882.

(17) Yen, Y.-C.; Schafer, C. T.; Gustavsson, M.; Eberle, S. A.; Dominik, P. K.; Deneka, D.; Zhang, P.; Schall, T. J.; Kossiakoff, A. A.; Tesmer, J. J. G.; Handel, T. M. Structures of Atypical Chemokine Receptor 3 Reveal the Basis for Its Promiscuity and Signaling Bias. *Sci. Adv.* **2022**, *8*, eabn8063.

(18) Maussang, D.; Mujić-Delić, A.; Descamps, F. J.; Stortelers, C.; Vanlandschoot, P.; Stigter-van Walsum, M.; Vischer, H. F.; van Roy, M.; Vosjan, M.; Gonzalez-Pajuelo, M.; van Dongen, G. A. M. S.; Merchiers, P.; van Rompaey, P.; Smit, M. J. Llama-Derived Single

Variable Domains (Nanobodies) Directed against Chemokine Receptor CXCR7 Reduce Head and Neck Cancer Cell Growth in Vivo. *J. Biol. Chem.* **2013**, *288* (41), 29562−29572.
(19) Tripathi, A.; Gaponenko, V.; Majetschak, M. Commercially Available Antibodies Directed against α-Adrenergic Receptor Subtypes and Other G Protein-Coupled Receptors with Acceptable Selectivity in Flow Cytometry Experiments. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2016**, *389* (2), 243−248.
(20) Stoddart, L. A.; Kilpatrick, L. E.; Hill, S. J. NanoBRET Approaches to Study Ligand Binding to GPCRs and RTKs. *Trends Pharmacol. Sci.* **2018**, *39* (2), 136−147.
(21) Iliopoulos-Tsoutsouvas, C.; Kulkarni, R. N.; Makriyannis, A.; Nikas, S. P. Fluorescent Probes for G-Protein-Coupled Receptor Drug Discovery. *Expert Opin. Drug Discovery* **2018**, *13* (10), 933−947.
(22) Dekkers, S.; Caspar, B.; Goulding, J.; Kindon, N. D.; Kilpatrick, L. E.; Stoddart, L. A.; Briddon, S. J.; Kellam, B.; Hill, S. J.; Stocks, M. J. Small-Molecule Fluorescent Ligands for the CXCR4 Chemokine Receptor. *J. Med. Chem.* **2023**, *66* (7), 5208−5222.
(23) Conroy, S.; Kindon, N. D.; Glenn, J.; Stoddart, L. A.; Lewis, R. J.; Hill, S. J.; Kellam, B.; Stocks, M. J. Synthesis and Evaluation of the First Fluorescent Antagonists of the Human P2Y 2 Receptor Based on AR-C118925. *J. Med. Chem.* **2018**, *61* (7), 3089−3113.
(24) *OEDOCKING 4.2.1.0*; OpenEye, Cadence Molecular Sciences Inc.: Santa Fe, NM, 2023; http://www.eyesopen.com.
(25) McGann, M. FRED and HYBRID Docking Performance on Standardized Datasets. *J. Comput. Aided. Mol. Des.* **2012**, *26* (8), 897−906.
(26) White, C. W.; Johnstone, E. K. M.; See, H. B.; Pfleger, K. D. G. NanoBRET Ligand Binding at a GPCR under Endogenous Promotion Facilitated by CRISPR/Cas9 Genome Editing. *Cell. Signal.* **2019**, *54*, 27−34.
(27) Zarca, A. M.; Adlere, I.; Viciano, C. P.; Arimont-Segura, M.; Meyrath, M.; Simon, I. A.; Paul Bebelman, J.; Laan, D.; Custers, J. H. G.; Janssen, E.; Versteegh, K. L.; Bosma, R.; de Esch, I. J. P.; Vischer, H. F.; Wijtmans, M.; Szpakowska, M.; Chevigné, A.; Hoffmann, C.; de Graaf, C.; Zarzycka, B. A.; Windhorst, A. D.; Smit, M. J.; Leurs, R. Pharmacological Characterization and Radiolabeling of VUF15485, a High-Affinity Small-Molecule Agonist for the Atypical Chemokine Receptor ACKR3 *bioRXiv* 2023, 2023.07.12.548622

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