# SUPPLEMENTARY MATERIAL

**A set of experimentally validated decoys for the human CC Chemokine Receptor 7 (CCR7) obtained by virtual screening**

**Matic Proj 1, Steven De Jonghe 2, Tom Van Loy 2, Marko Jukič 3,4, Anže Meden 1, Luka Ciber 5, Črtomir Podlipnik 5, Uroš Grošelj 5, Janez Konc 6, Dominique Schols 2, Stanislav Gobec 1,\***

1 University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ljubljana, Slovenia

2 KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Herestraat 49, box 1043, 3000 Leuven, Belgium

3 University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory of Physical Chemistry and Chemical Thermodynamics, Maribor, Slovenia.

4 University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia.

5 University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia

6 National Institute of Chemistry, Ljubljana, Slovenia

**\* Correspondence:**Stanislav Gobec  
[stanislav.gobec@ffa.uni-lj.si](mailto:stanislav.gobec@ffa.uni-lj.si)

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## FILTER configuration file

ELIMINATE\_METALS Sc,Ti,V,Cr,Mn,Fe,Co,Ni,Cu,Zn,Y,Zr,Nb,Mo,Tc,Ru,Rh,Pd,Ag,Cd

ALLOWED\_ELEMENTS H,C,N,O,F,P,S,Cl,Br,I

#-------------------------------------------------------------------

MIN\_MOLWT 200 "Minimum molecular weight"

MAX\_MOLWT 800 "Maximum molecular weight"

#-------------------------------------------------------------------

#Calculated LogP

MIN\_XLOGP -4.0 "Minimum XLogP"

MAX\_XLOGP 6.85 "Maximum XLogP"

#-------------------------------------------------------------------

AGGREGATORS true "Eliminate known aggregators"

PRED\_AGG true "Eliminate predicted aggregators"

#-------------------------------------------------------------------

#functional groups which often eliminate compounds from consideration

RULE 0 aldehyde

RULE 0 acid\_halide

RULE 0 peroxide

RULE 0 sulfonyl\_halide

RULE 0 carbonate

RULE 0 isonitrile

RULE 0 isocyanate

RULE 0 isothiocyanate

RULE 0 acyl\_cyanides

RULE 0 acylhydrazide

RULE 0 alphahalo\_amine

RULE 0 alphahalo\_ketone

RULE 0 anhydride

RULE 0 azide

RULE 0 aziridine

RULE 0 azocyanamides

RULE 0 oxaziridine

RULE 0 phosphoranes

RULE 0 dithioacetal

RULE 0 hydrazine

RULE 0 nitroso

RULE 0 triazine

RULE 0 disulfide

RULE 0 sulfinylthio

RULE 0 sulfonylnitrile

RULE 0 sulfinimine

RULE 0 fluorenylmethoxycarbonyl\_Fmoc

RULE 0 HOBT\_esters

RULE 0 trimethylsilyl\_TMS

RULE 0 cation\_C\_Cl\_I\_P\_or\_S

RULE 0 oxygen\_cation

RULE 0 lawesson\_s\_reagent

RULE 0 nonacylhydrazone

RULE 0 N\_methoyl

RULE 0 N\_P\_S\_Halides

RULE 0 NS\_beta\_halothyl

RULE 0 cytochalasin\_derivatives

RULE 0 squalestatin\_derivatives

RULE 0 monensin\_derivatives

RULE 0 iodine

RULE 0 iodoso

RULE 0 iodoxy

## Supporting figures



**Figure S1.** Known CCR7 antagonists that were used as a set of active compounds in the virtual screening campaign (Taveras et al., 2010; Jaeger et al., 2019).

## Supporting tables

**Table S1.** Compounds active on multiple chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, and CXCR7), used to construct a chemokine receptor targeted compound library.

|  |  |  |  |
| --- | --- | --- | --- |
| Maraviroc, CCR5 | Cenicriviroc, CCR2, CCR5 | PF-4136309, CCR2 | RS 504393,CCR2 |
| CCR1 antagonist 8, CCR1 | CCR2 antagonist 1, CCR2 | RS102895 hydrochloride, CCR1, CCR2 | BX471, CCR1 |
| Vercirnon, CCR9 | Cenicriviroc mesylate, CCR2, CCR5 | MK-0812 succinate, CCR2 | AZD2098, CCR4 |
| ZK756326 dihydrochloride, CCR8 | DAPTA, CCR5 | CCR2-RA-[R], CCR2 | BMS-813160, CCR2, CCR5 |
| MK-0812, CCR2 | GSK2239633A, CCR4 | Teijin compound 1, CCR2 | Aplaviroc, CCR5 |
| BX-471 hydrochloride, CCR1 | GW 766994, CCR3 | CCR5 antagonist 1, CCR5 | RS102895, CCR1, CCR2 |
| CCR3 antagonist 1, CCR3 | INCB3344, CCR2 | SB297006, CCR3 | TAK-779, CCR3, CCR5, CXCR3 |
| INCB 3284, CCR2 | TAK-220, CCR5 | Vicriviroc maleate, CCR5 | INCB 3284 dimesylate, CCR2 |
| CCR1 antagonist 1, CCR1 | AZD2423, CCR2 | YJC-10592, CCR2 | CCR251, CCR2 |
| CCR252, CCR2 | CCR253, CCR2 | JNJ27553292, CCR2 | CCR4Ant1, CCR4 |
| CCR4Ant2, CCR4 | CCR4Ant3, CCR4 | CCR4Ant11, CCR4 | CCR4Ant15, CCR4 |
| CCR4Ant22 | CCR4Ant23, CCR4 | CCR4Ast, CCR4 | AZD1678, CCR4 |
| AMD3100 (Plerixafor), CCR7, CXCR4 | Cosalane, CCR7 | Triptolide, CCR7 | Matrine, CCR7 |
| MLN3126, CCR9 | CCR9Ant31, CCR9 | CCR9Ant32, CCR9 | CCR9Ant33, CCR9 |
| CCR9Ant34, CCR9 | CCR9Ant35, CCR9 | Brintonamide D, CCR10 (ACKR2), CXCR7 | SB225002, CXCR2 |
| AZD 5096, CXCR2 | Danirixin, CXCR2 | Reparixin, CXCR1, CXCR2 | Ladarixin, CXCR2 |
| SX682, CXCR2 | MK7123, CXCR2 | CXCR2Ant5, CXCR2 | MSX122, CXCR4 |
| AMD3465, CXCR4 | KRH1636, CXCR4 | AMD11070, CXCR4 | KRH3955, CXCR4 |
| FC131, CXCR4 | IT1t, CXCR4 | Zach5, CXCR4 | Zach6, CXCR4 |
| Zach7, CXCR4 | Zach8, CXCR4 | Zach9, CXCR4 | CXCR7Ant1, CXCR7 |
| CXCR7Ant2, CXCR7 | CXCR7Ant3, CXCR7 | CXCR7Ant4, CXCR7 | CXCR7Ant5, CXCR7 |
| CXCR7Ant6, CXCR7 | CXCR7Ant7, CXCR7 | CXCR7Ant8, CXCR7 | CXCR7Ant9, CXCR7 |
| CXCR7Ant10, CXCR7 | CXCR7Ant11, CXCR7 | CXCR7Ant12, CXCR7 | CXCR7Ant13, CXCR7 |
| CXCR7Ant14, CXCR7 | CXCR7Ant15, CXCR7 | CXCR7Ant16, CXCR7 | CXCR7Ant17, CXCR7 |
| CXCR7Ant18, CXCR7 | CXCR7Ant19, CXCR7 | CXCR7Ant20, CXCR7 | CXCR7Ant21, CXCR7 |
| CXCR7Ant22, CXCR7 | CXCR7Ant23, CXCR7 | CXCR7Ant24, CXCR7 | CXCR7Ant25, CXCR7 |

## Chemistry – General Information

Compounds from libraries of commercially available compounds were obtained from various sources (AA Blocks, ChemBridge, ChemDiv, Enamine, Life Chemicals, Maybridge, Otava, Specs, UkrOrgSynthesis, and Vitas-M) and used as received. The purity of all compounds was above 90%, according to the suppliers. Purity of compounds from the FKKTlib academic compound library was determined to be above 90% (except for compound **C019**), see Supplementary Excel File.

The reagents and solvents were used as received from commercial suppliers. Reactions were monitored using analytical thin-layer chromatography (TLC) on silica gel 60 F254 Al plates. Developed plates were inspected under UV light and, if necessary, visualized with ninhydrin, vanillin/sulfuric acid, Dragendorff’s or potassium permanganate stains. Melting points were determined with Büchi 535 Melting Point Appartus (uncorrected). Nuclear magnetic resonance spectra were recorded on a Bruker Avance III 500 MHz and Bruker Avance III 400 MHz spectrometers at 500 MHz (400 MHz) for 1H, 126 MHz (101 MHz) for 13C, respectively, using DMSO-*d*6 or CDCl3 with TMS as the internal standard, as solvents. Chemical shifts are reported in *parts per million* (ppm), TMS peak was calibrated to 0 ppm or, alternatively, the central peak of the residual solvent resonance was used as the internal standard, *i.e.* for CDCl3 at 7.27 ppm for 1H and 77.16 ppm for 13C and DMSO-*d*6 at 2.50 ppm for 1H and 39.52 ppm for 13C, respectively. The multiplicities are reported as follows: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *m* (multiplet), *dd* (doublet of doublets), *ddd* (doublet doublet of doublets), *td* (triplet of doublets), *qd* (quartet of doublets), and *br* (broad), number of equivalent nuclei (by integration), coupling constants (*J*) quoted in Hertz (Hz). Mass spectra were recorded on Agilent 6224 Accurate Mass TOF LC/MS and Thermo Scientific Q Executive Plus LC-MS/MS spectrometers, and IR spectra on Thermo Nicolet FT-IR spectrophotometer. UHPLC analyses were performed on Thermo Scientific Dionex UltiMate 3000 modular system (Thermo Fisher Scientific Inc.) with Waters Acquity UPLC® HSS C18 SB column (2.1 × 50 mm, 1.8 µm) thermostated at 40 °C, injection volume, 1–5 µL; sample, 0.1–0.2 mg/mL in MeOH; flow rate, 0.4 mL/min; detector λ, 220 and 254 nm; mobile phase A: 0.1% TFA (v/v) in water; mobile phase B: MeCN. Method I: 0–5 min, 20%–100% B; 5–6 min, 20% B. Method II: 0–2 min, 0% B; 2–8 min, 0%–100% B. Method III: 0–2 min, 20% B; 2–5 min, 20%–90% B; 5–8 min, 90% B.

### 3-((2-((2-Methoxyethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)benzonitrile (**CS-2**)



3-((2-Ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)benzonitrile (Mejuch et al., 2017) (230 mg, 0.945 mmol) and 2-methoxyethylamine (1.1 eq., 90 µL) in acetonitrile (5 mL) were stirred for 18h at 60 °C. The reaction mixture was cooled in an icebath for 5 min, the precipitate was filtered, washed with ice-cold acetonitrile (2 x 5 mL), diethyl ether (30 mL) and dried in air to afford 3-((2-((2-methoxyethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)benzonitrile (Jaeger et al., 2019). Yield: 175 mg (0.645 mmol, 67.9%) of yellowish solid. mp = 242.6–243.8 °C. ESI-HRMS: m*/z* = 272.1028 (MH+); C14H14N3O3 requires: *m/z* = 272.1030 (MH+). *ν*max 3269, 3206, 3145, 3090, 3062, 2944, 2234, 1794, 1664, 1607, 1587, 1551, 1489, 1441, 1307, 1112, 1016, 864, 710, 630 cm–1. Purity: UPLC (method I, 254 nm): tr = 1.667 min, 96.1% total area. 1H NMR (400 MHz, DMSO-*d*6): *δ* 3.32 (*s*, 3H), 3.51 (*t*, *J* = 5.0 Hz, 2H), 3.75 – 3.81 (*m*, 2H), 7.45 (*dt*, *J* = 1.1, 7.5 Hz, 1H), 7.53 (*t*, *J* = 7.9 Hz, 1H), 7.61 – 7.66 (*m*, 1H), 7.89 (*s*, 1H), 7.93 (*s*, 1H), 9.94 (*s*, 1H). 13C NMR (101 MHz, DMSO-*d*6): *δ* 43.49, 58.06, 71.28, 112.15, 118.67, 120.81, 122.55, 125.81, 130.71, 139.99, 162.82, 169.49.





### Ethyl 4-((2-((2-methoxyethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)piperidine-1-carboxylate (**CS-3**)



Ethyl 4-((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)piperidine-1-carboxylate (Antane et al., 1996) was *in situ* prepared from ethyl 4-aminopiperidine-1-carboxylate (250 mg, 1.45 mmol) and diethyl squarate (1.1 eq., 236 µL) in absolute ethanol (2 mL). After 24h stirring at room temperature, 2-methoxyethylamine (1.5 eq., 190 µL) was added, and the stirring continued for 24h. Ethyl 4-((2-((2-methoxyethyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)piperidine-1-carboxylate (Jaeger et al., 2019) was isolated using reversed-phase column chromatography (RP-CC) (Isolera Biotage One Flash Chromatography system, SNAP Biotage KP-C18-HS column, 12 g) using a gradient of 0.1% TFA in deionized water and MeCN as eluent (gradient 10–100% MeCN in 15 column volumes (300 mL); 100% MeCN for 5 column volumes (100 mL)). After the RP-CC, fractions containing the product were combined, and the organic solvent removed *in vacuo*. The remaining aqueous solution was neutralized with saturated sodium bicarbonate solution and extracted with DCM (2 x 30 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered, and volatile components evaporated in vacuo to afford the pure product. Yield: 365 mg (1.12 mmol, 77.4%) of beige solid. mp = 157.1–158.4 °C. ESI-HRMS: *m/z* = 326.1707 (MH+); C15H24N3O5 requires: *m/z* = 326.1711 (MH+). *ν*max 3221, 2962, 1794, 1669, 1577, 1538, 1431, 1262, 1232, 1147, 1098, 1020, 788, 747, 700 cm–1. Purity: UPLC (method I, 254 nm): tr = 3.133 min, 97.1% total area. 1H NMR (400 MHz, CDCl3): *δ* 1.19 (*t*, *J* = 7.1 Hz, 3H), 1.47 – 1.59 (*m*, 2H), 1.88 – 1.97 (*m*, 2H), 2.89 (*t*, *J* = 10.1 Hz, 2H), 3.27 (*s*, 3H), 3.49 (*t*, *J* = 5.1 Hz, 2H), 3.73 – 3.81 (*m*, 2H), 3.96 – 4.14 (*m*, 5H), 7.62 (*s*, 1H), 7.88 (*s*, 1H). 13C NMR (101 MHz, CDCl3): *δ* 14.63, 32.84, 42.17, 44.12, 51.41, 58.75, 61.42, 71.84, 155.36, 167.50, 167.85, 181.72, 182.54.





## Supplementary references

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Mejuch, T., Garivet, G., Hofer, W., Kaiser, N., Fansa, E. K., Ehrt, C., et al. (2017). Small-Molecule Inhibition of the UNC119–Cargo Interaction. *Angew. Chem. Int. Ed.* 56, 6181–6186. doi:10.1002/anie.201701905.

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