Synthesis and Pharmacological Evaluation of Triazolopyrimidinone Derivatives as Noncompetitive, Intracellular Antagonists for CC Chemokine Receptors 2 and 5

*Natalia V. Ortiz Zacarías, Jacobus P. D. van Veldhoven, Lisa S. den Hollander, Burak Dogan, Joseph Openy, Ya-Yun Hsiao, Eelke B. Lenselink, Laura H. Heitman, Adriaan P. IJzerman**

Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research, Leiden University, P.O. Box 9502, 2300 RA, Leiden, The Netherlands

Table of contents:

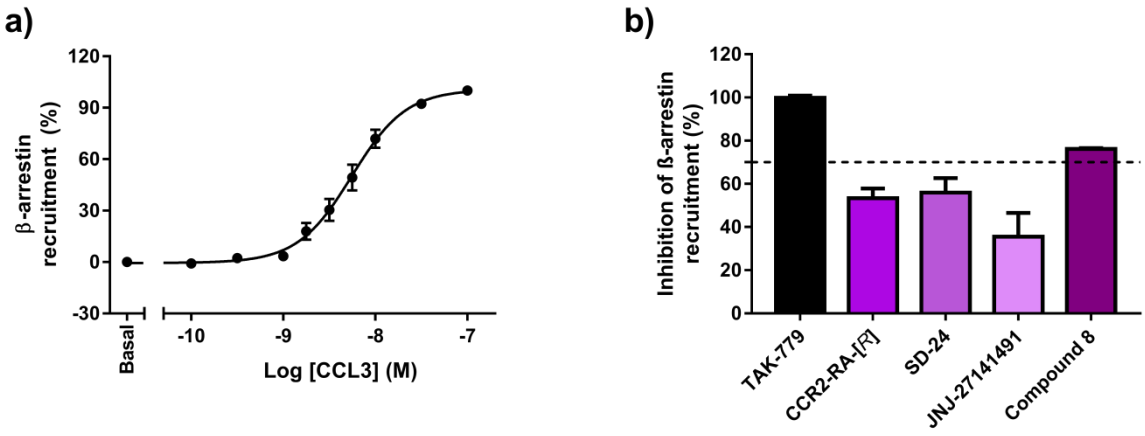

Figure S1. Characterization of intracellular ligands in a U2OS-CCR5 β-arrestin-recruitment assay. (a) Increasing concentrations of CCL3-induced β-arrestin recruitment in U2OS-CCR5 cells, with a pEC_{50} value of 8.3 ± 0.08 (6 nM) and a pEC_{80} of 7.9 ± 0.08 (14 nM). (b) Inhibition of β-arrestin recruitment in U2OS-CCR5 by the orthosteric compound TAK-779 and several intracellular ligands with different chemical structures, all tested at 1 μM, after stimulation with an EC_{80} concentration of CCL3. The dashed line indicates 70% inhibition. Only TAK-779 and compound **8** were able to inhibit CCL3-induced β-arrestin recruitment more than 70%.

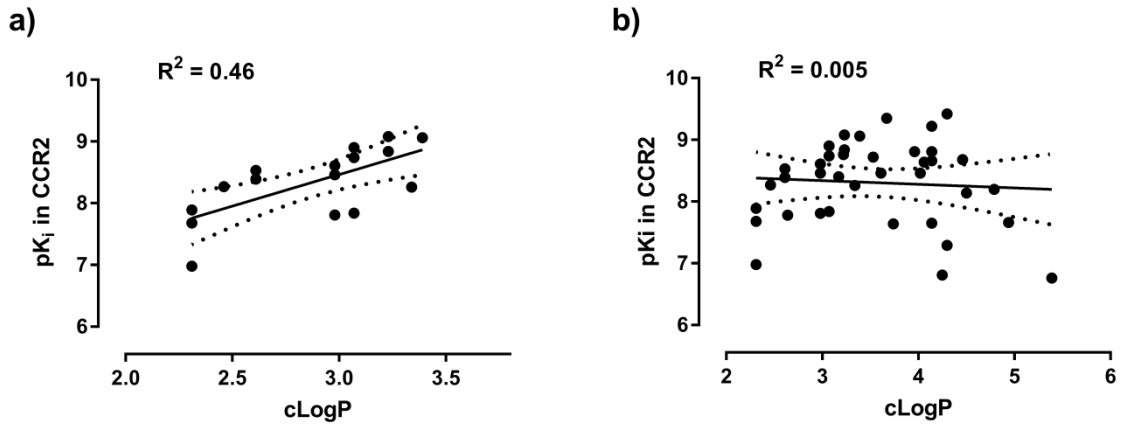

Figure S2. Correlation between log P (cLogP) and affinity (pK_i) values in CCR2. (a) Correlation shown for compounds **8** – **23** (Table 1), with R^1 modifications. (b) Correlation shown for all triazolopyrimidinone derivatives. In all cases, cLogP values were calculated using the calculator plugins in MarvinSketch, version 19.1.0, 2019, developed by ChemAxon (http://www.chemaxon.com). pK_i values were determined from [^{3}H]-CCR2-RA-[*R*] displacement assays in U2OS-CCR2 and are shown in Tables 1 – 3.

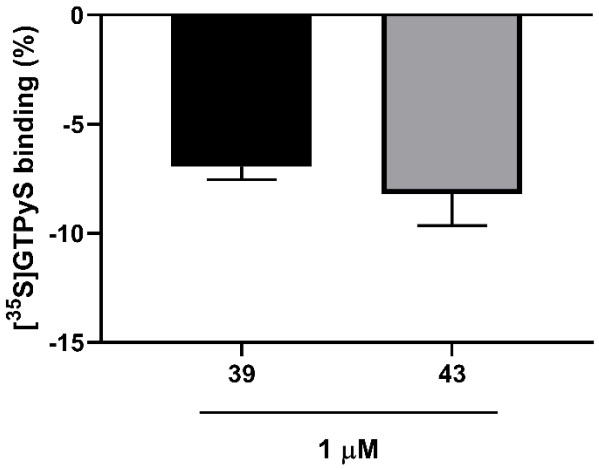

Figure S3. Characterization of compounds **39** and **43** as potential inverse agonists in hCCR2. In absence of CCL2, compounds **39** and **43** (1 μM) decrease basal [^{35}S]GTPγS binding levels by 6.9 ± 0.6% and 8.2 ± 1.5%, respectively. Data are presented as normalized mean ± SEM values of four experiments performed in triplicate, in which 0% represents basal activity and 100% represents [^{35}S]GTPγS binding after stimulation with 100 nM CCL2.

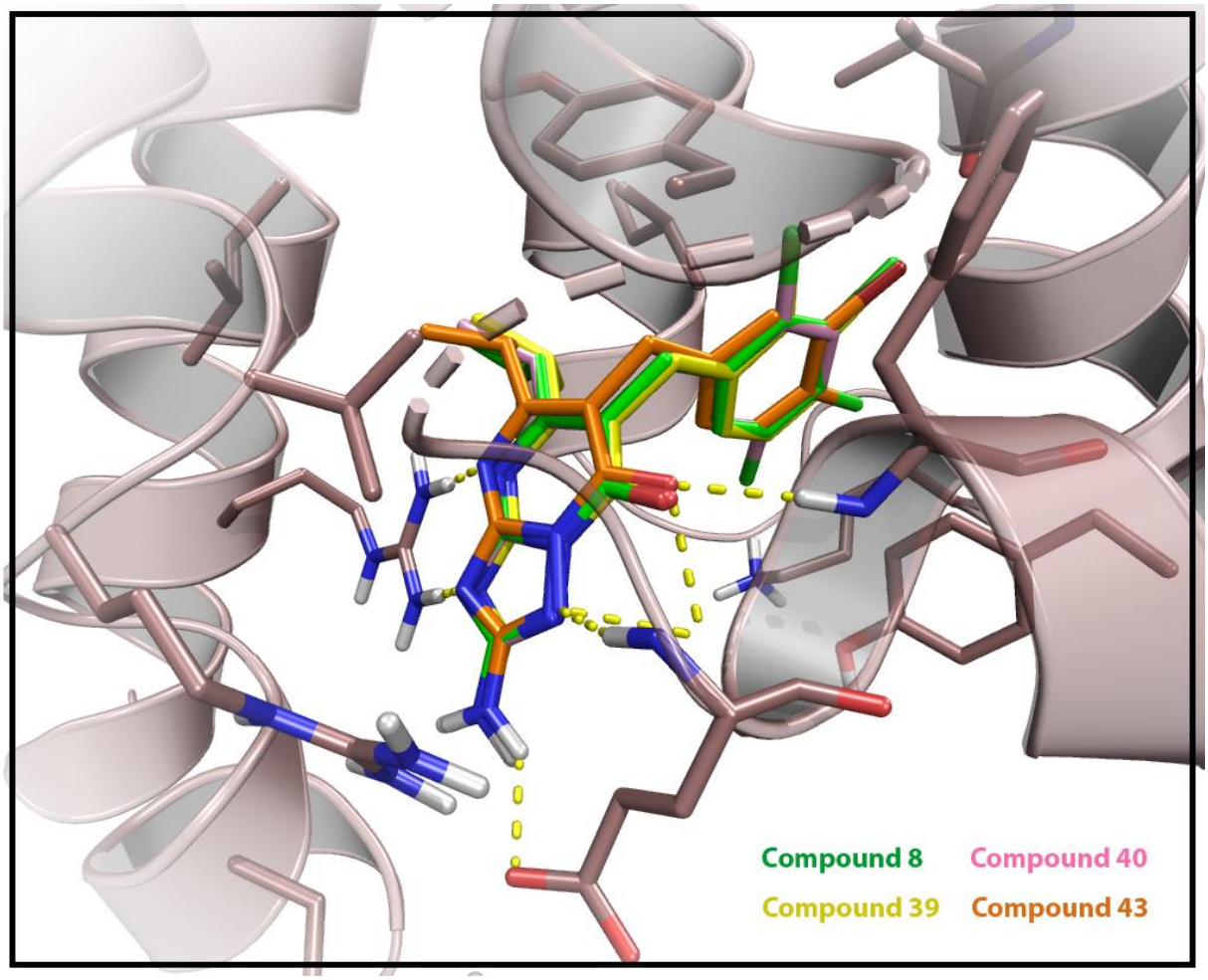

Figure S4. Docking of compounds **8**, **39**, **40** and **43**. Overlay showing the proposed binding mode of compounds **8** (green), **39** (yellow), **40** (pink) and **43** (orange) in hCCR2b. Model of hCCR2 is based on the crystal structure of CCR2 (PDB 5T1A).[1]

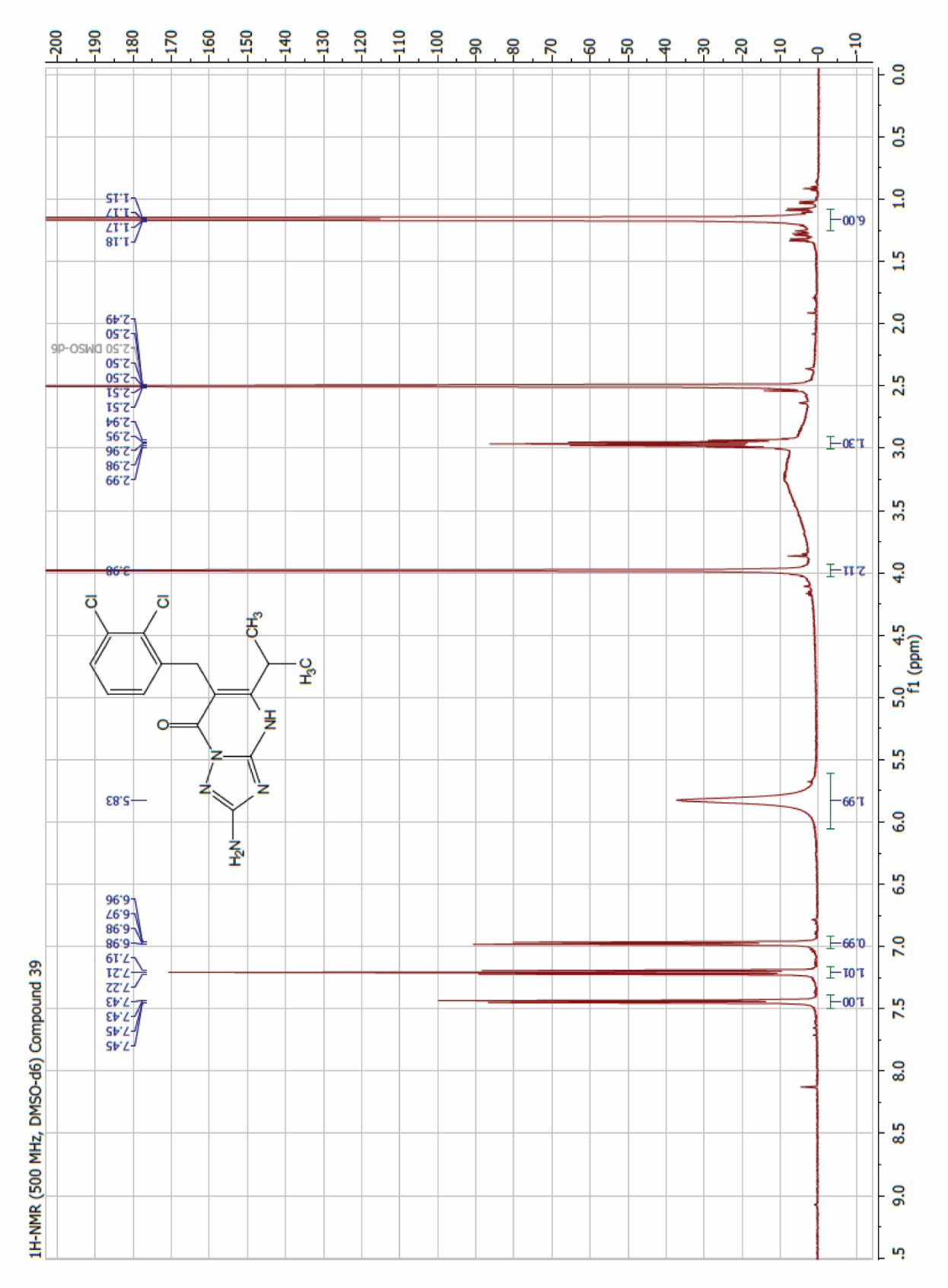

Figure S5. ^{1}H NMR of compound **39**, with peaks assigned.

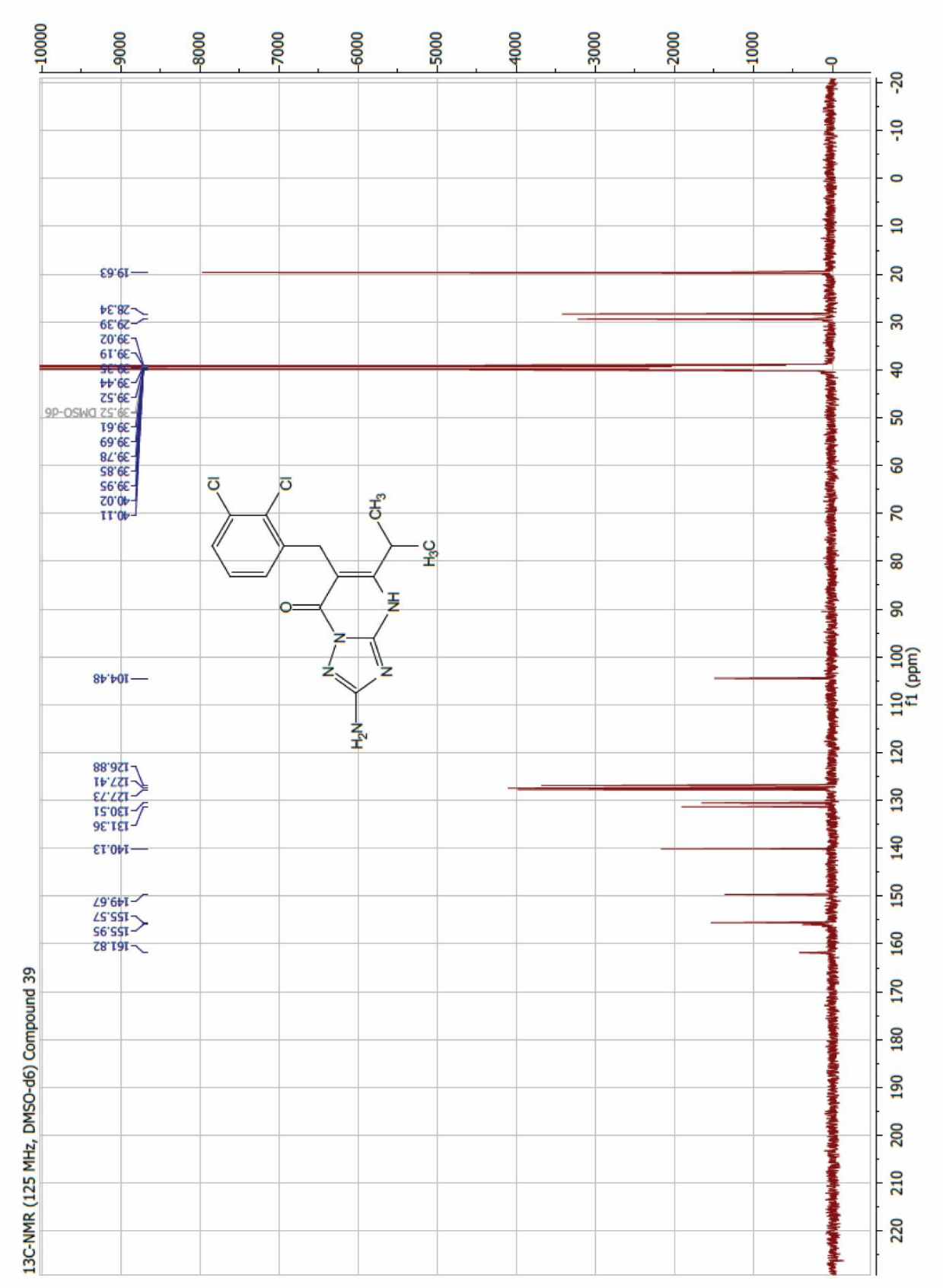

Figure S6. ^{13}C NMR of compound **39**, with peaks assigned.

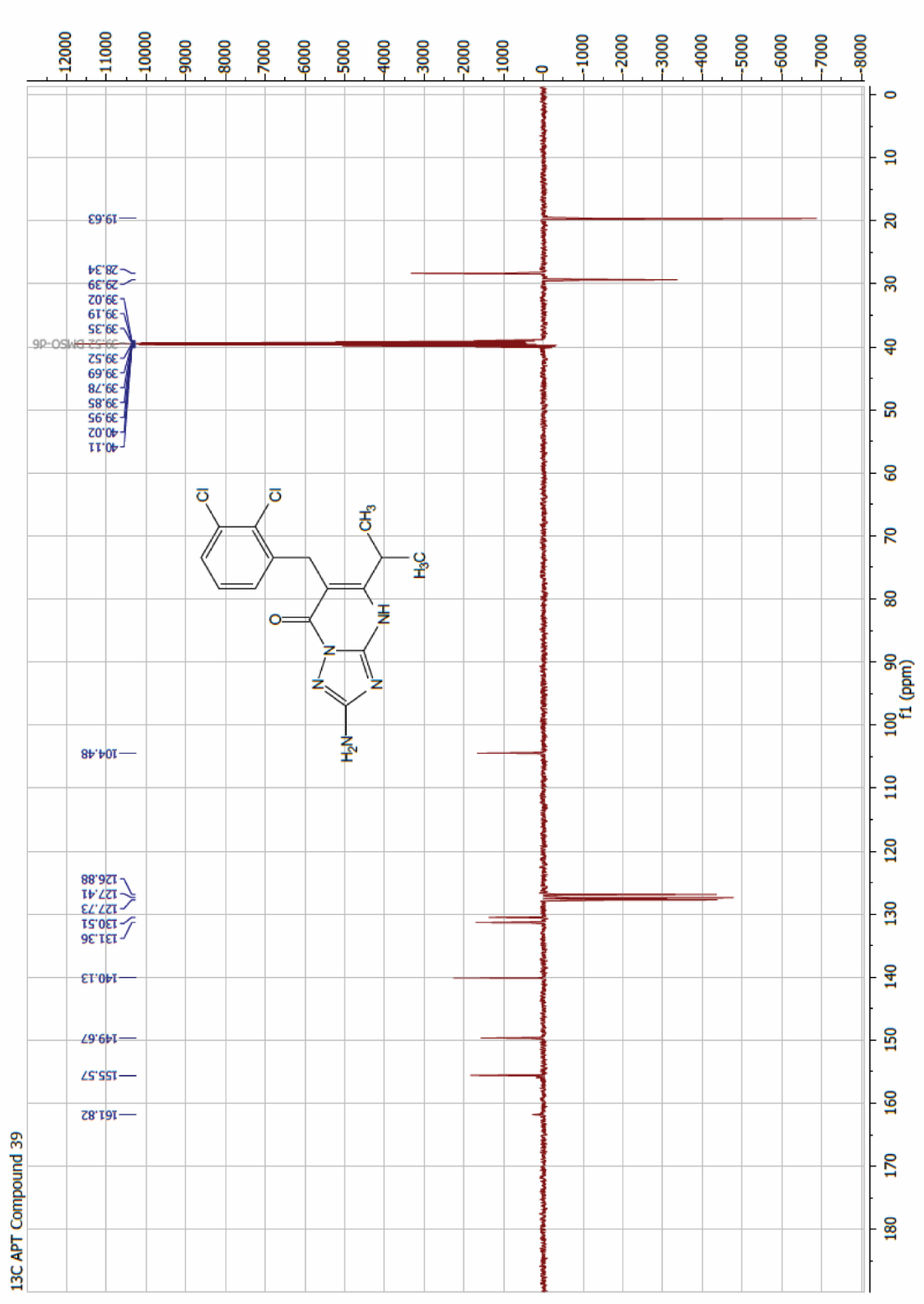

Figure S7. ^{13}C NMR-APT of compound **39**, with peaks assigned.

Table S1. List of intermediate compounds **4aa-na, 4bb-bq, 4eq-ev**.

Compound	R^3	R^1
4aa	Me	3-Cl
4ba	*c*Pr	3-Cl
4bb	*c*Pr	H
4bc	*c*Pr	2-Me
4bd	*c*Pr	2-Cl
4be	*c*Pr	2-OMe
4bf	*c*Pr	3-Me
4bg	*c*Pr	3-F
4bh	*c*Pr	3-Br
4bi	*c*Pr	3-I
4bj	*c*Pr	3-OMe
4bk	*c*Pr	3-CF3
4bl	*c*Pr	4-Me
4bm	*c*Pr	4-F
4bn	*c*Pr	4-Cl
4bo	*c*Pr	4-Br
4bp	*c*Pr	4-OMe
4bq	*c*Pr	3,4-diCl
4ca	Et	3-Cl
4da	Pr	3-Cl
4ea	*i*Pr	3-Cl
4eq	*i*Pr	3,4-diCl
4er	*i*Pr	2,3-diCl
4es	*i*Pr	2,5-diCl
4et	*i*Pr	3,5-diCl
4eu	*i*Pr	3,5-diBr
4ev	*i*Pr	3-Br, 4-Cl
4fa	Bu	3-Cl
4ga	2-EtBu	3-Cl
4ha	Pent	3-Cl
4ia	*c*Pent	3-Cl
4ja	Hex	3-Cl
4ka	Hept	3-Cl
4la	Ph	3-Cl
4ma	4-MePh	3-Cl
4na	CH_2CH_2Ph	3-Cl

Table S2. Functional activity of TAK-779 and CCR2-RA-[*R*] in hCCR5, using a CCL3-induced β-arrestin recruitment assay.

Compound	**pIC_{50} ± SEM (IC_{50}, nM)**	**Hill slope**
TAK-779	8.32 ± 0.17 (6)	-1.1 ± 0.1
CCR2-RA-[*R*]	6.15 ± 0.02 (703)	-2.4 ± 0.2**

Data represent the mean ± standard error of the mean (SEM) of three independent experiments performed in duplicate. **$p < 0.01$ ($p = 0.0038$) versus Hill slope (n_H) of TAK-779, determined with a two-tailed, unpaired Student's t-test.

Table S3. Functional activity of compounds **8**, **39** and **43** in hCCR2, using a CCL2-induced β-arrestin recruitment assay.

Compound	pIC_{50} ± SEM (IC_{50}, nM)	Hill slope
8	7.99 ± 0.01 (10)	-2.7 ± 0.2
39	7.68 ± 0.05 (21)	-2.5 ± 0.2
43	8.40 ± 0.01 (4)	-3.4 ± 0.4

Data represent the mean ± standard error of the mean (SEM) of three independent experiments performed in duplicate.

References:

1. Zheng, Y.; Qin, L.; Ortiz Zacarías, N. V.; de Vries, H.; Han, G. W.; Gustavsson, M.; Dabros, M.; Zhao, C.; Cherney, R. J.; Carter, P.; Stamos, D.; Abagyan, R.; Cherezov, V.; Stevens, R. C.; IJzerman, A. P.; Heitman, L. H.; Tebben, A.; Kufareva, I.; Handel, T. M. Structure of CC chemokine receptor 2 with orthosteric and allosteric antagonists. *Nature* **2016,** 540, 458-461.