

Integrating chemical and genetic silencing strategies to identify host kinase-phosphatase inhibitor networks that control bacterial infection.

Harald M.H.G. Albers,^{‡,§,1} Coenraad Kuijl,^{‡,1} Jeroen Bakker,[‡] Loes Hendrickx,^{‡,†} Sharida Wekker,[‡] Nadha Farhou,[‡] Nora Liu,[‡] Bernat Blasco-Moreno,[#] Tiziana Scanu,[‡] Jeroen den Hertog,[°] Patrick Celie,[∇] Huib Ovaa,^{,‡,§} and Jacques Neefjes^{*,‡,§}*

[‡]Division of Cell Biology, [§]Netherlands Proteomics Centre, [∇]Division of Biochemistry, the Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

[#]Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Doctor Aiguader, 88, 08003 Barcelona, Spain

[°] Hubrecht Institute, Utrecht, The Netherlands.

[†] Deceased.

¹ Equal contribution

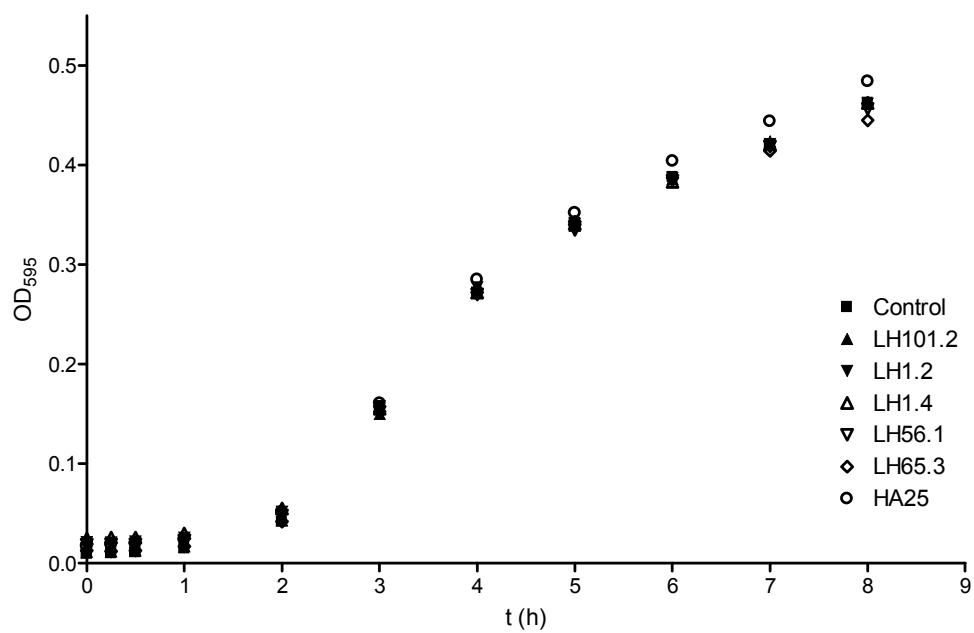
Corresponding authors:

Huib Ovaa: h.ovaa@nki.nl

Jacques Neefjes: j.neefjes@nki.nl

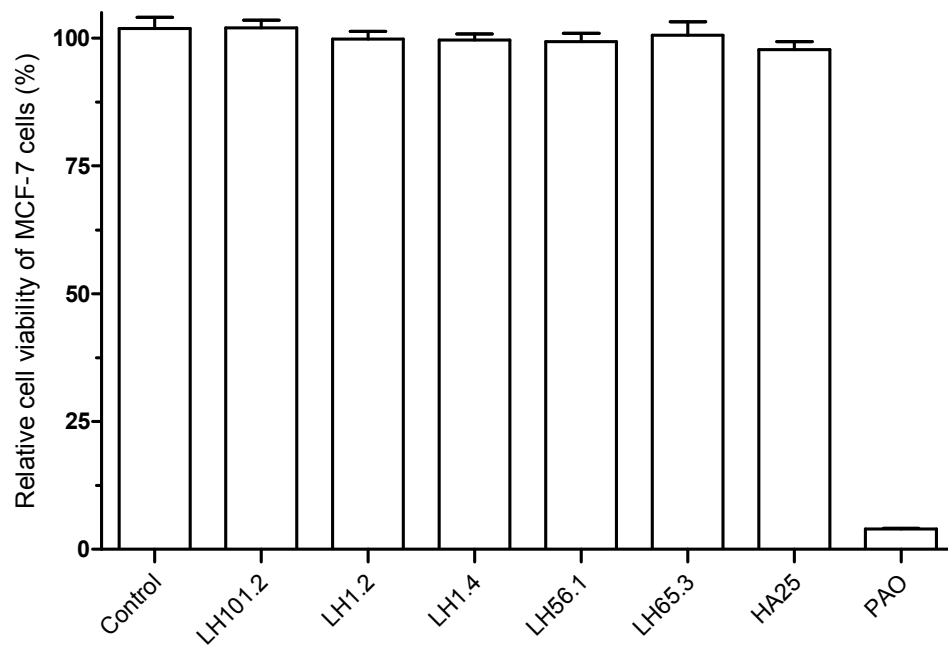
Table of contents

| | | |
|------------------------------------------------------------------------|----|----------------|
| Effect of compounds on <i>S. typhimurium</i> growth in LB medium | S2 | Supp. Fig. S1 |
| Effect of compounds on cell viability of MCF-7 cells | S3 | Supp. Fig. S2 |
| Effect of compounds on Phospho-p44/42 MAPK levels | S4 | Supp. Fig. S3 |
| Selectivity studies for LH65.3 | S5 | Supp. Fig. S4 |
| List of screened phosphatase and phosphatase-like siRNAs | S6 | Supp. Table S1 |
| Synthesis of the inhibitor library | S8 | |
| Spectral data LH65.3 (HPLC-MS, ¹ H and ¹³ C NMR) | S9 | |



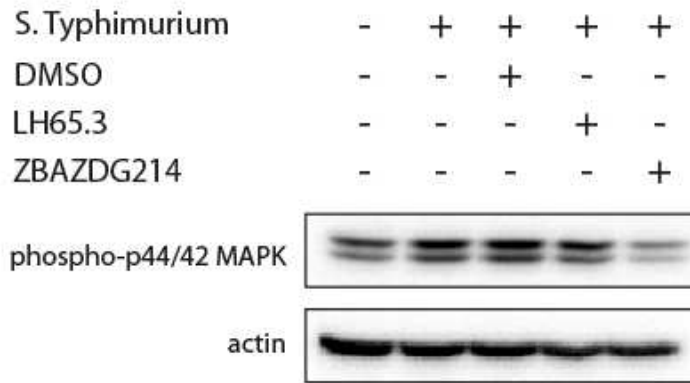
Supplementary Figure S1: Effect of compounds on *S. typhimurium* growth in LB medium.

Bacterial growth of *S. typhimurium* was measured in the presence or absence of 10 μ M of compound at a wavelength of 595 nm. Data points have not been fitted for clarity.

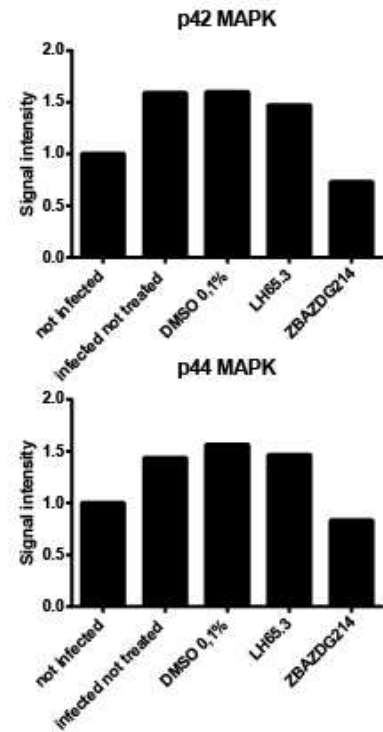


Supplementary Figure S2: Effect of compounds on cell viability of MCF-7 cells. Cell viability of MCF-7 cells was tested using the conditions of the *S. typhimurium* FACS assay. Compounds were incubated for 42 h at a concentration of 10 μ M. Phenylarsine oxide (PAO) was used as a control for cell death.

A.

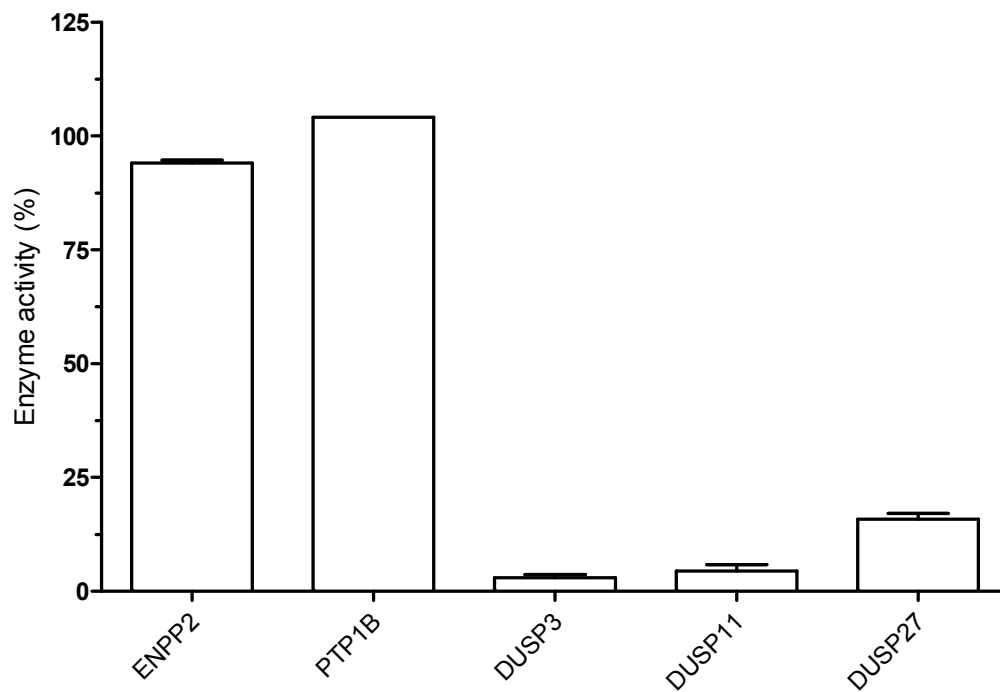


B.



Supplementary Figure S3: Effect of compounds on Phospho-p44/42 MAPK levels after *S. typhimurium* infection. MCF7 cells were pre-treated with LH65,3 (5 μ M) or MEK inhibitor ZBAZDG214 (5 μ M) before infection. 10 Hours post infection (in presence of the compounds), cells were lysed in a RIPA buffer containing phosphatase inhibitors.

(a) Phospho-p44/42 MAPK levels were analyzed by Western blotting (antibody: Cell Signalling #9101) and actin was stained as a loading control. (b) Signal intensity for p44/42 MAPK and for actin were determined by Image Lab v3.0 software and the ratio of p42 (top) or p44 (bottom) over actin determined. Values are relative to “not infected” sample.



Supplementary Figure S4: Selectivity studies for LH65.3. Selectivity for LH65.3 has been evaluated for ENPP2, a lipid phosphodiesterase which is prone to inhibition of thiazolidine-2,4-dione pharmacophores. In addition, selectivity has been tested against PTP1B, the founding member of the protein tyrosine phosphatase (PTP) family. Shown is mean + S.D of 3 or more experiments; PTP1B, n=1.

Supplementary Table S1: List of screened phosphatase and phosphatase-like siRNAs

| Name | Catalog number | Locus id | Accession id | Name | Catalog number | Locus id | Accession id |
|----------|----------------|----------|--------------|----------|----------------|----------|--------------|
| ENPP2 | M-004601-01 | 5168 | NM_006209 | DUSP13 | M-007887-00 | 51207 | NM_016364 |
| PTPN6 | M-009778-00 | 5777 | NM_002831 | DUSP14 | M-007888-00 | 11072 | NM_007026 |
| C7ORF16 | M-018324-01 | 10842 | NM_006658 | DUSP15 | M-008484-01 | 128853 | NM_080611 |
| CDKN3 | M-003879-00 | 1033 | NM_005192 | DUSP18 | M-007891-00 | 150290 | NM_152511 |
| DUSP1 | M-003484-02 | 1843 | NM_004417 | DUSP19 | M-007892-01 | 142679 | NM_080876 |
| DUSP10 | M-003965-01 | 11221 | NM_007207 | DUSP21 | M-007893-01 | 63904 | NM_022076 |
| DUSP2 | M-003565-01 | 1844 | NM_004418 | DUSP23 | M-007909-00 | 54935 | NM_017823 |
| DUSP22 | M-004517-00 | 56940 | NM_020185 | MGC1136 | M-008027-01 | 78986 | NM_024025 |
| DUSP4 | M-003963-02 | 1846 | NM_001394 | MGC26484 | M-008030-00 | 168448 | XM_171149 |
| DUSP5 | M-003566-01 | 1847 | NM_004419 | MK-STYX | M-008031-01 | 51657 | NM_016086 |
| DUSP6 | M-003964-01 | 1848 | NM_001946 | MTMR1 | M-008037-01 | 8776 | NM_003828 |
| DUSP7 | M-003567-00 | 1849 | XM_037430 | MTMR2 | M-008038-00 | 8898 | NM_016156 |
| DUSP8 | M-003568-00 | 1850 | NM_004420 | MTMR4 | M-008040-01 | 9110 | NM_004687 |
| ILKAP | M-010260-00 | 80895 | NM_030768 | MTMR7 | M-008041-00 | 9108 | XM_044727 |
| PFKFB1 | M-006761-00 | 5207 | NM_002625 | PDP2 | M-022572-00 | 57546 | NM_020786 |
| PFKFB2 | M-006762-01 | 5208 | NM_006212 | PPEF1 | M-009479-00 | 5475 | NM_006240 |
| PFKFB3 | M-006763-00 | 5209 | NM_004566 | PPEF2 | M-012250-01 | 5470 | NM_006239 |
| PFKFB4 | M-006764-00 | 5210 | NM_004567 | PPM1B | M-008281-01 | 5495 | NM_002706 |
| PPP1R1B | M-012745-00 | 84152 | NM_032192 | PPM1E | M-008964-00 | 22843 | NM_014906 |
| PPP2CA | M-003598-00 | 5515 | NM_002715 | PPM1F | M-009544-00 | 9647 | NM_014634 |
| PPP4C | M-008486-01 | 5531 | NM_002720 | PPM1G | M-005264-01 | 5496 | NM_002707 |
| PTPN5 | M-003600-01 | 84867 | NM_032781 | PPM1L | M-008679-00 | 151742 | NM_139245 |
| PTPRG | M-008069-00 | 5793 | NM_002841 | PPM2C | M-008718-00 | 54704 | NM_018444 |
| PTPRJ | M-008476-01 | 5795 | NM_002843 | PPP1CA | M-008927-00 | 5499 | NM_002708 |
| PTPRR | M-004017-01 | 5801 | NM_002849 | PPP1CB | M-008685-00 | 5500 | NM_002709 |
| PTPRT | M-008072-01 | 11122 | NM_007050 | PPP2R1A | M-010259-01 | 5518 | NM_014225 |
| TRIO | M-005047-00 | 7204 | NM_007118 | PPP2R5A | M-009352-01 | 5525 | NM_006243 |
| ACYP2 | M-008864-00 | 98 | NM_138448 | PPP2R5B | M-009366-00 | 5526 | NM_006244 |
| PPP1R16B | M-004065-00 | 26051 | NM_015568 | PPP2R5C | M-009433-00 | 5527 | NM_002719 |
| PPP2R3A | M-017376-00 | 5523 | NM_002718 | PPP2R5D | M-009799-01 | 5528 | NM_006245 |
| SAG | M-011105-00 | 6295 | NM_000541 | PPP2R5E | M-008531-01 | 5529 | NM_006246 |
| ZFHX1B | M-006914-00 | 9839 | NM_014795 | PPP3CC | M-010005-00 | 5533 | NM_005605 |
| ACP5 | M-009615-01 | 54 | NM_001611 | PPP5C | M-009259-00 | 5536 | NM_006247 |
| PPP3CA | M-008300-01 | 5530 | NM_000944 | PPP6C | M-009935-01 | 5537 | NM_002721 |
| PSPH | M-011888-01 | 5723 | NM_004577 | PTP4A1 | M-006333-01 | 7803 | NM_003463 |
| ACPI | M-019058-00 | 52 | NM_004300 | PTP4A2 | M-009078-00 | 8073 | NM_003479 |
| ACPP | M-009262-00 | 55 | NM_001099 | PTP4A3 | M-006859-01 | 11156 | NM_007079 |
| CDC14A | M-003469-00 | 8556 | NM_003672 | PTPDC1 | M-008584-00 | 138639 | NM_152422 |
| CDC14B | M-003470-02 | 8555 | NM_003671 | PTPLA | M-008742-00 | 9200 | NM_014241 |
| CDC25A | M-003226-02 | 993 | NM_001789 | PTPN1 | M-003529-04 | 5770 | NM_002827 |
| CDC25B | M-003227-02 | 994 | NM_004358 | PTPN11 | M-003947-01 | 5781 | NM_002834 |
| CDC25C | M-003228-01 | 995 | NM_001790 | PTPN12 | M-008064-01 | 5782 | NM_002835 |
| DUSP11 | M-007885-00 | 8446 | NM_003584 | PTPN13 | M-008065-00 | 5783 | NM_006264 |
| EPM2A | M-006896-01 | 7957 | NM_005670 | PTPN21 | M-009379-01 | 11099 | NM_007039 |
| PPAP2B | M-017312-01 | 8613 | NM_003713 | PTPN22 | M-008066-00 | 26191 | NM_012411 |
| PPAP2C | M-011500-00 | 8612 | NM_003712 | PTPN23 | M-009417-00 | 25930 | NM_015466 |
| PPM1D | M-004554-00 | 8493 | NM_003620 | PTPN3 | M-009372-01 | 5774 | NM_002829 |
| PPP1R3C | M-017077-00 | 5507 | NM_005398 | PTPN4 | M-009489-01 | 5775 | NM_002830 |
| PTEN | M-003023-01 | 5728 | NM_000314 | PTPN7 | M-008394-00 | 5778 | NM_002832 |
| PTPN14 | M-008509-00 | 5784 | NM_005401 | PTPN9 | M-008832-00 | 5780 | NM_002833 |
| PTPRU | M-009328-01 | 10076 | NM_005704 | PTPRA | M-004519-00 | 5786 | NM_002836 |
| TPTE2 | M-008107-00 | 93492 | NM_130785 | PTPRB | M-004994-02 | 5787 | NM_002837 |
| CTDP1 | M-009326-01 | 9150 | NM_004715 | PTPRC | M-008067-00 | 5788 | NM_002838 |
| DUSP12 | M-007886-01 | 11266 | NM_007240 | PTPRD | M-008527-00 | 5789 | NM_002839 |

| Name | Catalog number | Locus id | Accession id | Name | Catalog number | Locus id | Accession id |
|--------------|----------------|----------|--------------|----------|----------------|----------|--------------|
| PTPRE | M-008068-02 | 5791 | NM_006504 | C21ORF6 | M-013856-00 | 10069 | NM_016940 |
| PTPRF | M-008375-01 | 5792 | NM_002840 | DUSP3 | M-007894-00 | 1845 | NM_004090 |
| PTPRH | M-009448-00 | 5794 | NM_002842 | ALPI | M-008673-00 | 248 | NM_001631 |
| PTPRK | M-004204-01 | 5796 | NM_002844 | TPTE | M-008745-01 | 7179 | NM_013315 |
| PTPRM | M-006326-00 | 5797 | NM_002845 | ENPP5 | M-009805-01 | 59084 | NM_021572 |
| PTPRN | M-009315-01 | 5798 | NM_002846 | PON2 | M-009676-00 | 5445 | NM_000305 |
| PTPRN2 | M-008070-00 | 5799 | NM_002847 | IMPA2 | M-008348-00 | 3613 | NM_014214 |
| ACP2 | M-008205-00 | 53 | NM_001610 | PPP2R2A | M-004824-01 | 5520 | NM_002717 |
| INPP5B | M-021811-01 | 3633 | NM_005540 | OCRL | M-010026-01 | 4952 | NM_000276 |
| ALPPL2 | M-003455-01 | 251 | NM_031313 | ENPP7 | M-009059-00 | 339221 | NM_178543 |
| PPP3R1 | M-009869-01 | 5534 | NM_000945 | PTPRS | M-009662-01 | 5802 | NM_002850 |
| IGBP1 | M-011298-01 | 3476 | NM_001551 | SSH3 | M-008937-00 | 54961 | NM_017857 |
| ANP32E | M-015844-00 | 81611 | NM_030920 | FBP2 | M-010139-01 | 8789 | NM_003837 |
| LPPR4 | M-009911-00 | 9890 | NM_014839 | PPAP2A | M-019098-00 | 8611 | NM_003711 |
| PPP2CB | M-003599-02 | 5516 | NM_004156 | INPP1 | M-008505-01 | 3628 | NM_002194 |
| ENPP1 | M-003809-01 | 5167 | NM_006208 | PR48 | M-019459-00 | 28227 | NM_013239 |
| MAP3K7IP1 | M-004770-00 | 10454 | NM_006116 | PIB5PA | M-009108-00 | 27124 | NM_014422 |
| ACP6 | M-008665-00 | 51205 | NM_016361 | TENS1 | M-009997-00 | 64759 | NM_022748 |
| INPP5D | M-003013-01 | 3635 | NM_005541 | C14ORF24 | M-018433-00 | 283635 | NM_173607 |
| DUT | M-010258-00 | 1854 | NM_001948 | STYX | M-009571-01 | 6815 | NM_145251 |
| SYNJ1 | M-019486-01 | 8867 | NM_003895 | PTPN18 | M-009385-00 | 26469 | NM_014369 |
| PPP1R11 | M-011917-01 | 6992 | NM_021959 | RNGTT | M-009782-00 | 8732 | NM_003800 |
| DKFZP761G058 | M-018772-00 | 152926 | NM_152542 | FHIT | M-004952-01 | 2272 | NM_002012 |
| FLJ40125 | M-009006-00 | 147699 | NM_178494 | SYNJ2 | M-012624-00 | 8871 | NM_003898 |
| AKAP11 | M-009277-01 | 11215 | NM_016248 | PPP1CC | M-006827-00 | 5501 | NM_002710 |
| BPNT1 | M-008664-01 | 10380 | NM_006085 | PME-1 | M-005211-00 | 51400 | NM_016147 |
| PPM1A | M-009574-01 | 5494 | NM_021003 | CILP | M-008295-00 | 8483 | NM_003613 |
| ACPT | M-008366-01 | 93650 | NM_033068 | FLJ23751 | M-008557-00 | 92370 | NM_152282 |
| PHPT1 | M-016904-00 | 29085 | NM_014172 | FRMPD2 | M-008854-00 | 143162 | NM_152428 |
| ENPP3 | M-004540-00 | 5169 | NM_005021 | MINPP1 | M-009705-00 | 9562 | NM_004897 |
| PTPN2 | M-008969-00 | 5771 | NM_002828 | PTPRZ1 | M-009685-00 | 5803 | NM_002851 |
| PPP1R7 | M-019589-00 | 5510 | NM_002712 | INPP4B | M-011539-00 | 8821 | NM_003866 |
| LOC151242 | M-023104-00 | 151242 | XM_087137 | ALPL | M-008658-00 | 249 | NM_000478 |
| HSPC129 | M-008272-00 | 51496 | NM_016396 | PPP1R2 | M-015361-00 | 5504 | NM_006241 |
| MTM1 | M-008036-01 | 4534 | NM_000252 | I-4 | M-012962-00 | 80316 | NM_025210 |
| ALPP | M-003454-01 | 250 | NM_001632 | RWDD2 | M-015117-00 | 112611 | NM_033411 |
| MTMR3 | M-008039-01 | 8897 | NM_021090 | DNAJC6 | M-009885-00 | 9829 | NM_014787 |
| ENPP4 | M-009214-01 | 22875 | NM_014936 | ENPP6 | M-008704-00 | 133121 | NM_153343 |
| PON1 | M-009229-00 | 5444 | NM_000446 | FBP1 | M-008725-00 | 2203 | NM_000507 |
| IMPA1 | M-010172-01 | 3612 | NM_005536 | PPP2R2B | M-003022-01 | 5521 | NM_004576 |
| PPP1R8 | M-010903-00 | 5511 | NM_002713 | PTPRO | M-008500-01 | 5800 | NM_002848 |
| PPP2R4 | M-005214-00 | 5524 | NM_021131 | | | | |

Synthesis of the inhibitor library

General. Chemicals were obtained from Sigma-Aldrich and used without further purification unless otherwise noted. For isolation by centrifugation a Heraeus Multifuge 3_{S-R} centrifuge was used. Products were spun at 4400 x g, at 298 K for 5 min.

Nuclear magnetic resonance spectra (¹H and ¹³C NMR) were determined in deuterated dimethyl sulfoxide (d₆-DMSO) using a Bruker Avance 300 (¹H: 300 MHz; ¹³C: 75 MHz) at 298 K, unless indicated otherwise. Peakshapes are indicated with the symbols ‘d’ (doublet), ‘dd’ (double doublet), ‘s’ (singlet), ‘bs’ (broad singlet) and ‘m’ (multiplet). Chemical shifts (δ) are given in ppm and coupling constants *J* in Hz. Dimethyl sulfoxide (δ_H = 2.50 ppm; δ_C = 39.51 ppm) was used as internal reference.

LC-MS measurements were performed on a system equipped with a Waters 2795 Separation Module (Alliance HT), Waters 2996 Photodiode Array Detector (190-750 nm), Atlantis[®] T3 C18 column (2.1x100 mm, 3 μm) and an LCT[™] Orthogonal Acceleration Time of Flight Mass Spectrometer. Samples were run at a flowrate of 0.40 mL min⁻¹ at 313 K, using gradient elution (water/acetonitrile/formic acid) from 950/50/1 (v/v/v) to 50/950/1 (v/v/v).

The purity of all tested compounds was determined by LC-MS analyses and was greater than 95%, unless otherwise stated.

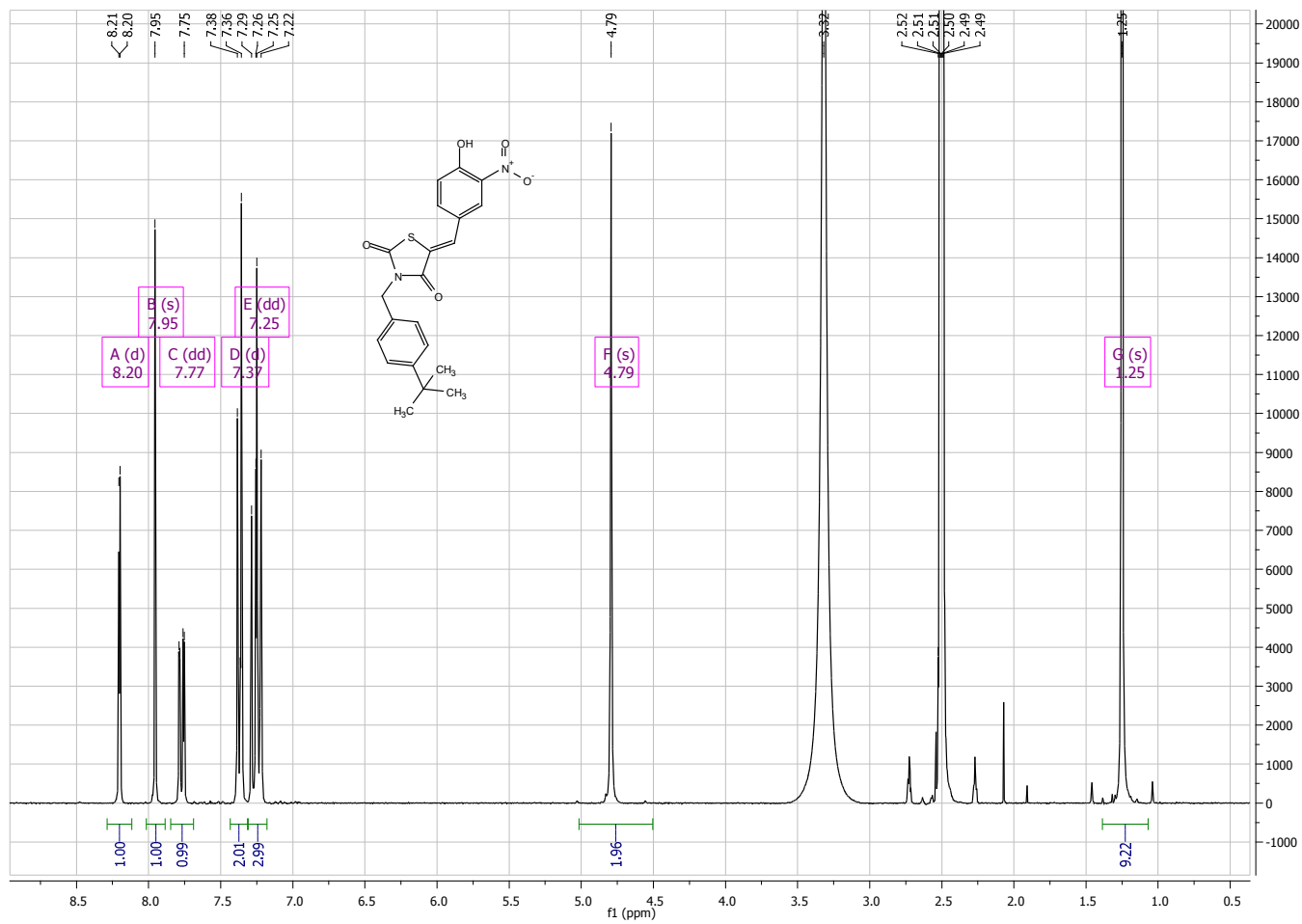
Synthesis of the inhibitor library. In short, to a cooled solution (277 K) of thiazolidine-2,4-dione (5.87 g, 50 mmol) in DMF (100 ml) sodium hydride (60% in oil, 1.8 g, 45 mmol) was added. A solution of the appropriate benzyl bromide (36.8 mmol) in DMF (25 ml) was added to the reaction mixture. The mixture was allowed to warm up to room temperature and was stirred for 4 h. Then the mixture was poured into of ice water (250 ml) and hexane (100 ml) was added. After a night at 277 K the precipitated crystals were filtrated and dried to give *N*-alkylated thiazolidine-2,4-dione.

The resulting *N*-alkylated thiazolidine-2,4-dione (0.317 mmol) was dissolved in ethanol (2.5 mL) containing piperidine (70 μL, 0.709 mmol) and the appropriate aldehyde (0.348 mmol) was added and the solution was refluxed overnight. Upon cooling to room temperature the product precipitated out of solution. Centrifugation and washing with ethanol gave homogeneous compound.

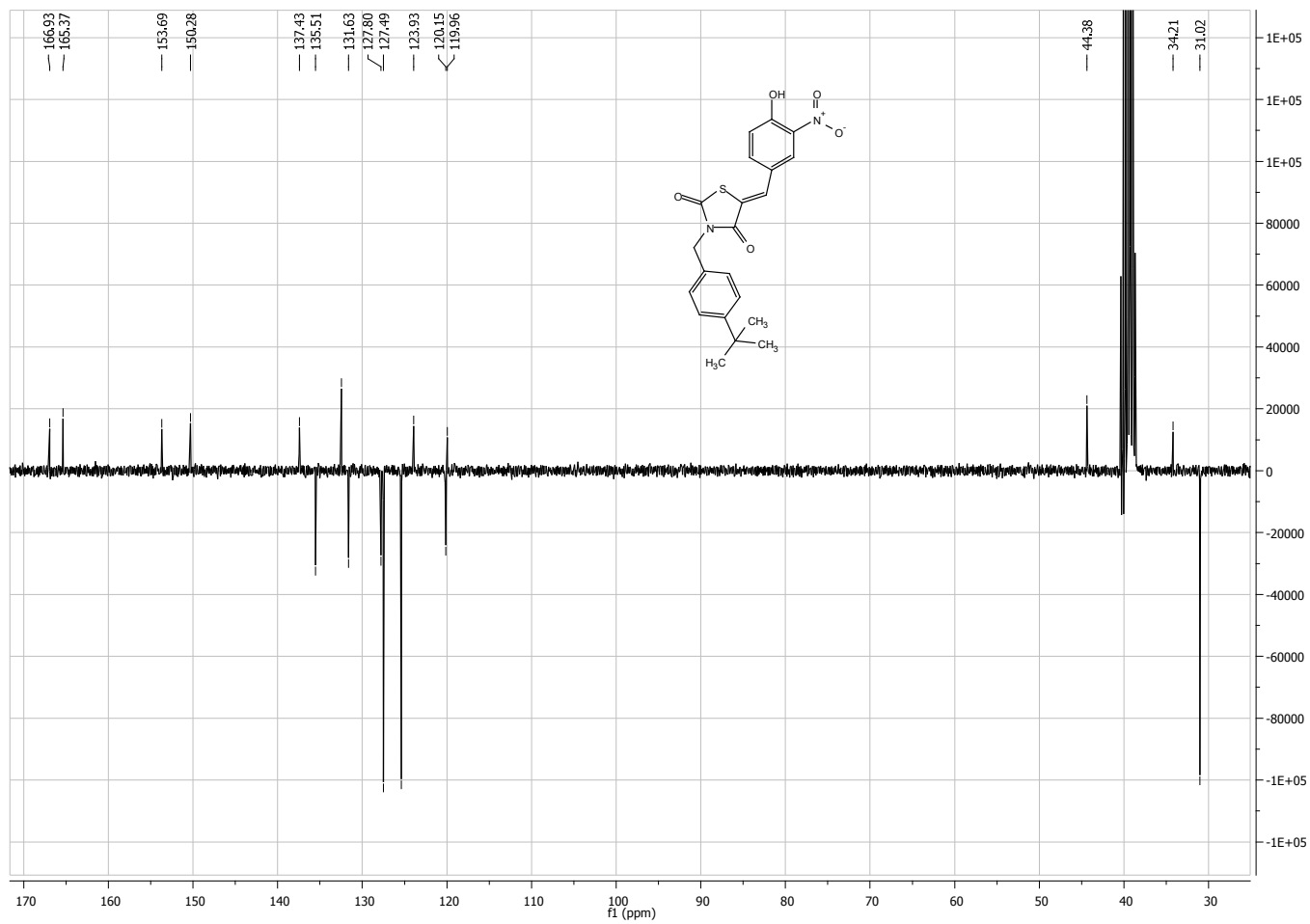
Spectral data on LH65.3

(HPLC-MS, ^1H and ^{13}C NMR)

(Z)-3-(4-(tert-butyl)benzyl)-5-(4-hydroxy-3-nitrobenzylidene)thiazolidine-2,4-dione (LH65.3); ¹H NMR



(Z)-3-(4-(tert-butyl)benzyl)-5-(4-hydroxy-3-nitrobenzylidene)thiazolidine-2,4-dione (LH65.3); ¹³C NMR



(Z)-3-(4-(tert-butyl)benzyl)-5-(4-hydroxy-3-nitrobenzylidene)thiazolidine-2,4-dione (LH65.3); HPLC-MS

