

## **SUPPLEMENTARY INFORMATION**

### **Small molecule screening to reveal mechanisms underlying aquaporin-2 trafficking**

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**Running title: Small molecules affecting AQP2**

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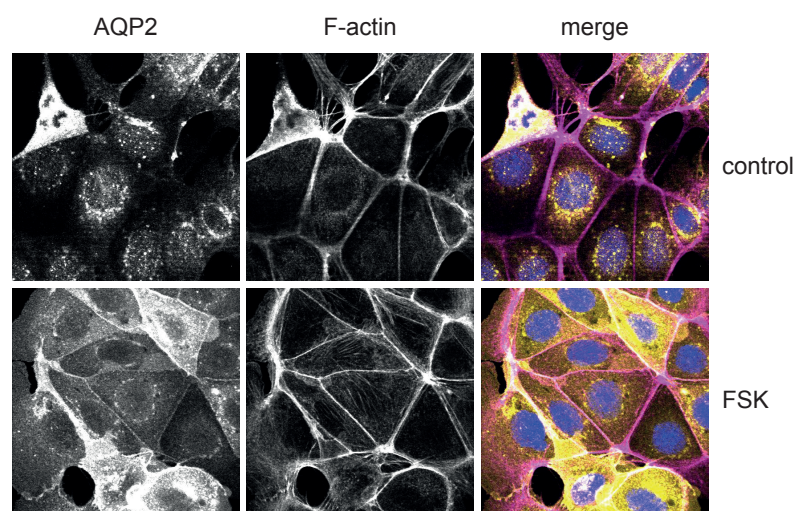
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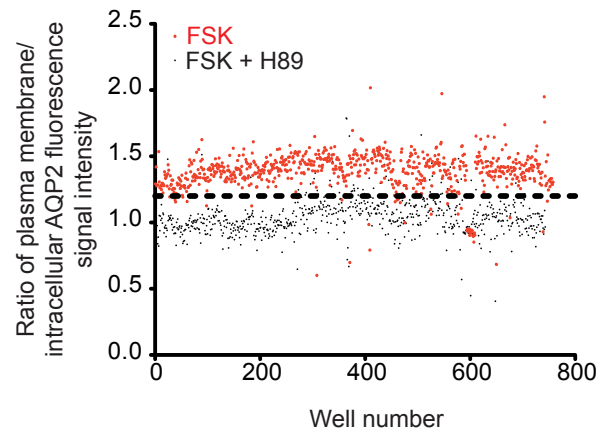
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Suppl. Fig.1

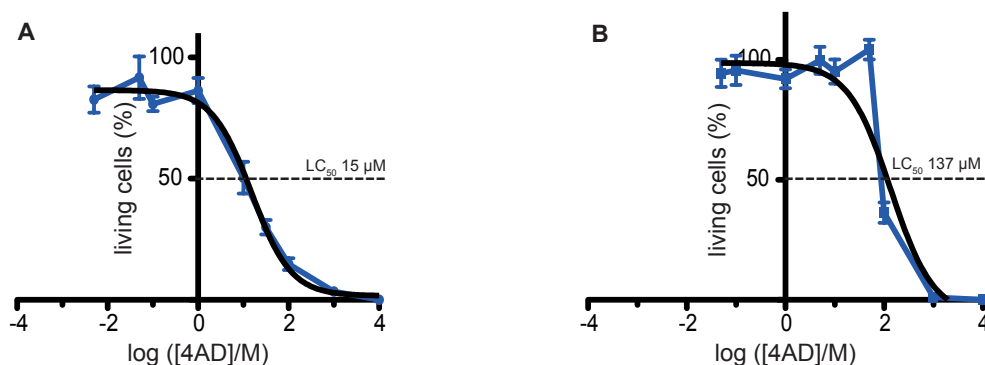


Detection of AQP2 and F-actin in mouse collecting duct cells stably expressing human AQP2 (MCD4 cells). MCD4 cells were treated with forskolin (FSK 10  $\mu$ M, 20 minutes) or left untreated. Cells were fixed with paraformaldehyde, permeabilized with Triton X-100 and AQP2 was detected by immunofluorescence microscopy with specific antibody H27 (Maric et al. AJP, 1998) and Cy3-coupled anti-rabbit secondary antibodies (yellow). F-actin was detected by TRITC-Phalloidin staining (magenta). Nuclei were stained with DAPI (blue). Fluorescence signals were visualized using a laser-scanning microscope (LSM 510 META, 100x magnification). Shown are representative images from three independent experiments.

Suppl. Fig. 2

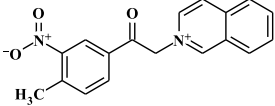
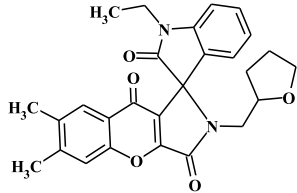
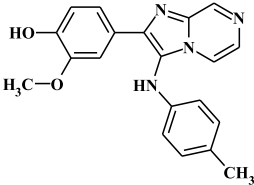
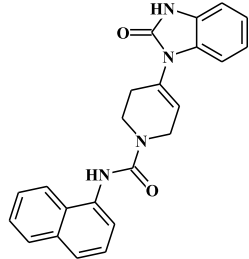
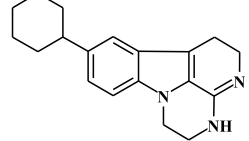
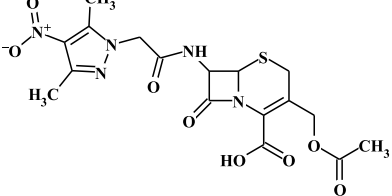
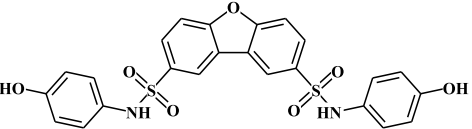


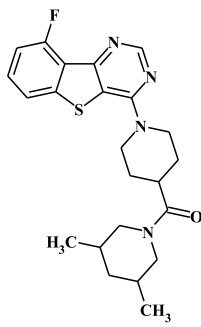
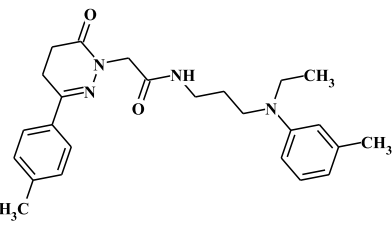
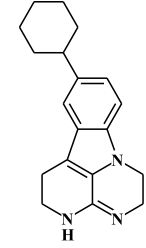
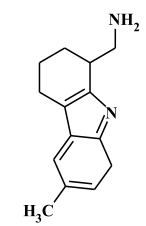
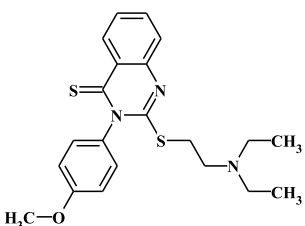
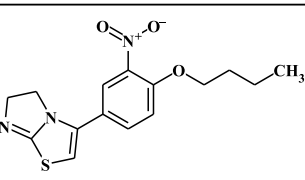
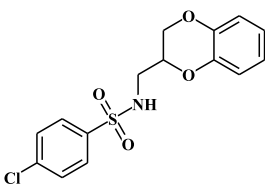
The localization of AQP2 in MCD4 cells in the presence of forskolin (FSK) or the combination of FSK and the PKA inhibitor H89. Primary screening was carried out in 384 well plates of MCD4 cells with 32 wells of controls in each plate, 16 wells treated with FSK alone (10  $\mu$ M, 20 min) and 16 wells treated with a combination of FSK and H89 (30  $\mu$ M, 100 min; see also Figs. 1A and B). The localization of AQP2 was expressed as ratio of fluorescence signal intensities at the plasma membrane to intracellular fluorescence signal intensity. The ratio determined in cells treated with FSK =  $1.40 \pm 0.1$ , for cells treated with the combination of FSK and H89 it was  $0.91 \pm 0.1$ . Based on these values, ratios  $\leq 1.2$  were considered low plasma membrane abundance (dashed line). Treatment of MCD4 cells with forskolin in the presence of 83 of the library compounds resulted in ratios  $\leq 1.2$ , indicating that they inhibited the FSK-induced AQP2 redistribution (see Suppl. Tab. 1).

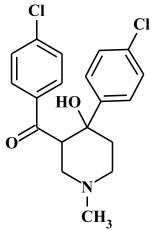
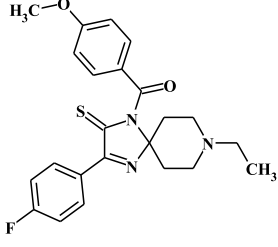
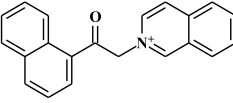
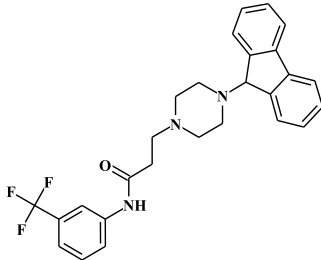
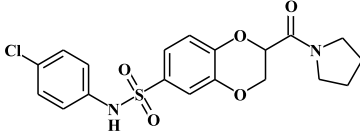
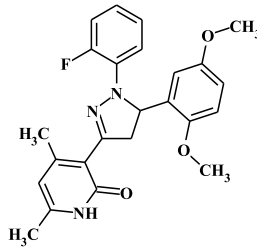
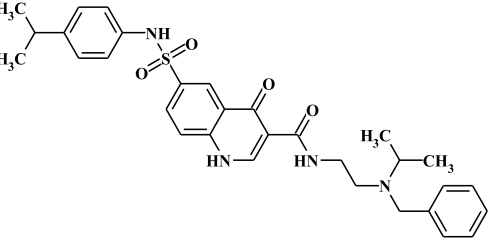


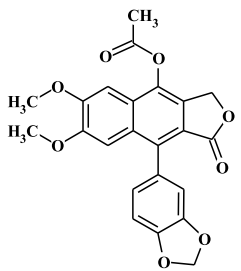
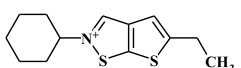
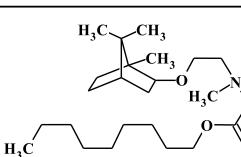
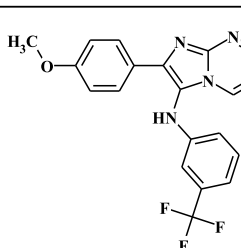
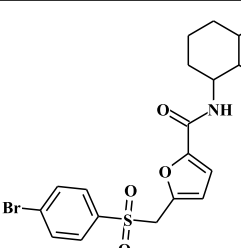
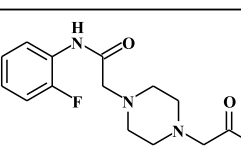
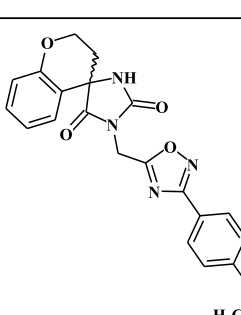
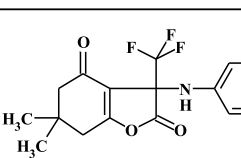
Determination of  $LC_{50}$  values for 4AD in MCD4 and IMCD cells. Cytotoxicity was evaluated using the MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MCD4 (A) or primary IMCD cells (B) were seeded into 96 well plates, grown for 24 hours and left either untreated or incubated with 4AD in concentrations of 1 mM, 100, 50, 10, 1, 0.1, 0.05 and 0.05  $\mu$ M for 24 hours. The 4AD-containing incubation medium was replaced by a solution of MTT in medium (0.5 mg/ml) followed by additional 4 hours of incubation. Metabolically active living cells reduce yellow water-soluble tetrazolium salt to violet water-insoluble formazan crystals. MTT solution was removed and cells were lysed with a mixture of DMSO, SDS and acetic acid (14.9 ml, 1.5g, 90 $\mu$ l) to dissolve formazan crystals. Absorbance at 595 nm wavelengths was determined using an EnSpire plate reader (PerkinElmer Inc., Rodgau, Germany). Values from untreated cells were referred to as 100 % vitality. Shown are averages from three independent experiments (mean  $\pm$  SEM, quadruplicates per condition).  $LC_{50}$  values of 15  $\mu$ M and 137  $\mu$ M were determined for MCD4 and primary IMCD cells, respectively (Suppl. Fig. 2A and 2B). The reason for the 10fold difference is unclear. IMCD cells seem generally less susceptible for uptake of exogenous molecules than other cell types. For example, inhibition of Rho family members with bacterial toxins such as toxin B requires also 10fold higher concentrations than used for other cell types (Klussmann, E, Tamma, G, Lorenz, D, Wiesner, B, Maric, K, Hofmann, F, Aktories, K, Valenti, G, Rosenthal, W: An inhibitory role of Rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. The Journal of biological chemistry, 276: 20451-20457, 2001).

Suppl. Tab. 1

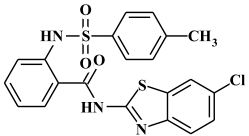
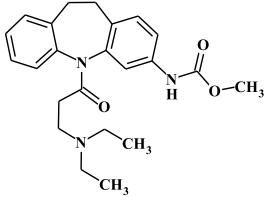
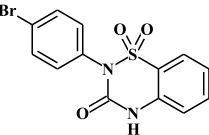
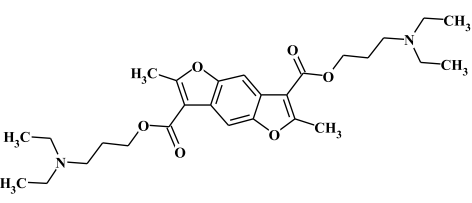
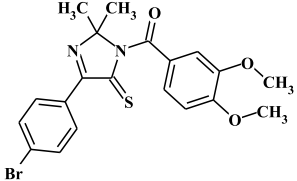
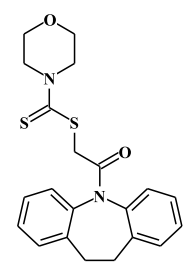
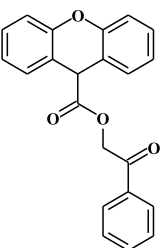
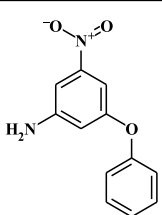
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| 201196 | <br><chem>Cc1ccc(cc1[N+](=O)[O-])C(=O)CNc2cccc3ccccc23</chem><br>$C_{18}H_{15}N_2O_3$           | 0.23  | 72    | G14  | 307.3 | -0.51 | 3      | 0      |
| 214625 | <br><chem>Cc1cc2c(c3cc(C)c(C)c3O2)C(=O)N(Cc4ccccc4)C(=O)c5ccccc5</chem><br>$C_{27}H_{26}N_2O_5$ | 0.54  | 110   | B5   | 458.5 | 3.19  | 5      | 0      |
| 212086 | <br><chem>Cc1ccc(cc1)Nc2nc3ccccc3n2c4ccc(O)c(OC)c4</chem><br>$C_{20}H_{18}N_4O_2$               | 0.57  | 103   | K8   | 346.4 | 3.16  | 6      | 2      |
| 214590 | <br><chem>Cc1ccc(cc1)Nc2cc3ccccc3n2C(=O)Nc4ccccc4</chem><br>$C_{23}H_{20}N_4O_2$               | 0.69  | 110   | K18  | 384.4 | 3.24  | 2      | 2      |
| 205831 | <br><chem>Cc1ccc(cc1)Nc2cc3ccccc3n2C(=O)Nc4ccccc4</chem><br>$C_{19}H_{23}N_3$                 | 0.75  | 85    | N5   | 293.4 | 3.62  | 2      | 1      |
| 214775 | <br><chem>Cc1ccc(cc1)Nc2cc3ccccc3n2C(=O)Nc4ccccc4</chem><br>$C_{17}H_{19}N_5O_8S$             | 0.76  | 110   | N20  | 453.4 | -1.36 | 8      | 2      |
| 205653 | <br><chem>Cc1ccc(cc1)Nc2cc3ccccc3n2C(=O)Nc4ccccc4</chem><br>$C_{24}H_{18}N_2O_7S_2$           | 0.78  | 85    | I5   | 510.5 | 3.52  | 6      | 4      |

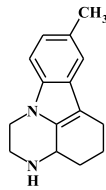
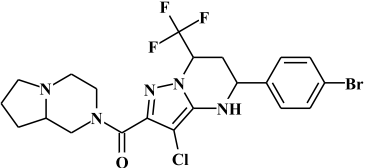
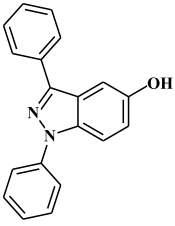
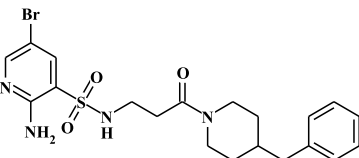
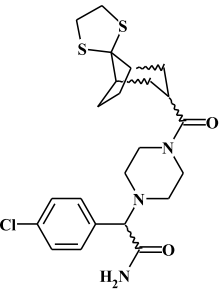
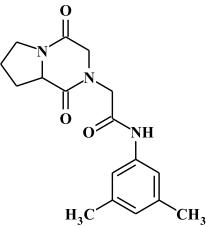
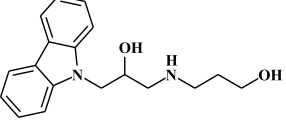
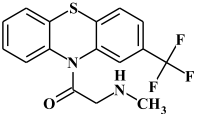
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| 214336 | <br><chem>C23H27FN4OS</chem>    | 0.79 | 109 | P19 | 426.6  | 4.55 | 4 | 0 |
| 216086 | <br><chem>C25H32N4O2</chem>     | 0.79 | 114 | L17 | 420.5  | 3.47 | 4 | 1 |
| 214645 | <br><chem>C19H23N3</chem>       | 0.8  | 110 | J9  | 293.4  | 3.62 | 2 | 1 |
| 200277 | <br><chem>C14H18N2</chem>      | 0.83 | 69  | J4  | 214.31 | 1.22 | 2 | 1 |
| 205839 | <br><chem>C21H25N3OS2</chem>  | 0.83 | 85  | N7  | 399.6  | 5.23 | 3 | 0 |
| 206893 | <br><chem>C15H17N3O3S</chem>  | 0.83 | 88  | J7  | 319.4  | 3.07 | 5 | 0 |
| 216478 | <br><chem>C15H14ClNO4S</chem> | 0.83 | 115 | L6  | 339.8  | 2.78 | 4 | 1 |

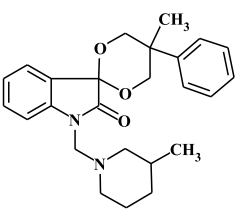
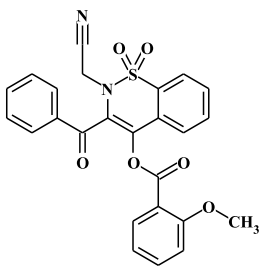
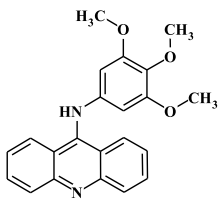
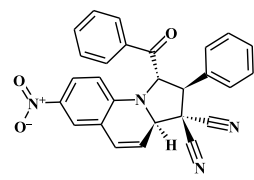
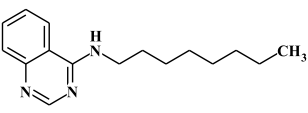
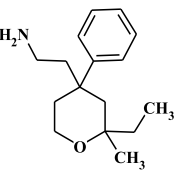
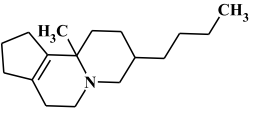
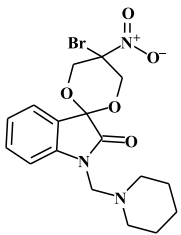
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| 212678 |    | 0.84 | 105 | K1  | 425.5 | 4.99 | 4 | 0 |
| 200728 |    | 0.86 | 71  | O5  | 298.4 | 0.02 | 1 | 0 |
| 202659 |   | 0.86 | 76  | F5  | 465.5 | 5.25 | 3 | 1 |
| 215591 |  | 0.86 | 113 | M4  | 422.9 | 2.31 | 5 | 1 |
| 200014 |  | 0.88 | 69  | K3  | 421.5 | 4.09 | 5 | 1 |
| 212634 |  | 0.88 | 104 | D14 | 560.7 | 5.2  | 6 | 3 |

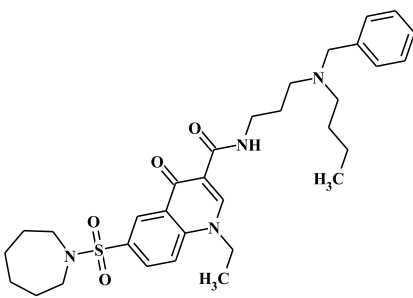
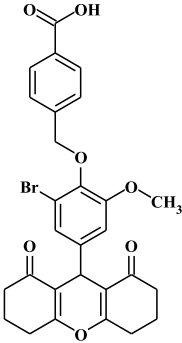
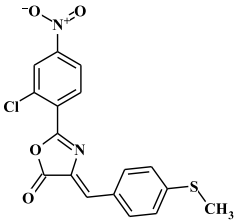
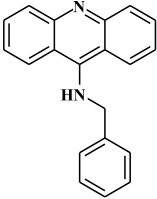
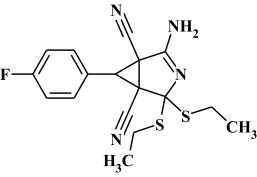
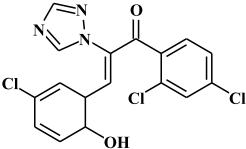
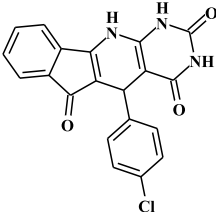
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|-------------------------------------|--|------|------|-----|-------|------|---|---|
| 4-acetydiphillin<br>(4AD)<br>216407 | <br><chem>CC(=O)Oc1c2c(c3cc(OC)c(OC)c3c1O2)C4=CC=C5OCOC5=C4</chem>        | 0.88 | 115  | N9  | 422.4 | 3.08 | 6 | 0 |
| 203240                              | <br><chem>CCc1c2sc3ccccc3n2s1</chem>                                      | 0.89 | 78   | O17 | 252.4 | 1.6  | 0 | 0 |
| 202254                              | <br><chem>CCCCCCCCCOC(=O)CN(C)[CH+]1CCCC1</chem>                          | 0.9  | 75   | K14 | 410.7 | 1.66 | 2 | 0 |
| 212248                              | <br><chem>COc1ccc(cc1)-c2nc3ccncc3n2Nc4cc(F)(F)cc4</chem>                 | 0.9  | 103  | P4  | 384.4 | 4.09 | 5 | 1 |
| 215223                              | <br><chem>BrC1=CC=C(C=C1)S(=O)(=O)C2=CC=C3OC(=O)N3C2c4ccc5ccccc45</chem> | 0.9  | 112  | M21 | 474.4 | 4.23 | 3 | 1 |
| 216332                              | <br><chem>Fc1ccccc1C(=O)N2CCN(CC2)CC(=O)Nc3ccc(Cl)c(C(F)(F)F)c3</chem>  | 0.91 | 115  | G14 | 472.9 | 2.84 | 4 | 2 |
| 100358                              | <br><chem>COc1ccc(cc1)C2=CC=C3C(=O)N(C2=O)C(=O)N3Cc4nc5ccccc5n4</chem>  | 0.91 | 1002 | K1  | 404.4 | 3.46 | 5 | 1 |
| 208264                              | <br><chem>Fc1ccccc1C(F)(F)F2C(=O)N2C(=O)C3=CC=CC=C3C2=O</chem>          | 0.92 | 92   | O20 | 339.3 | 3.18 | 3 | 1 |

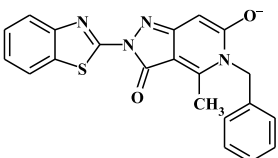
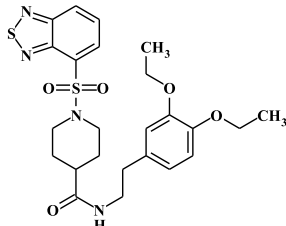
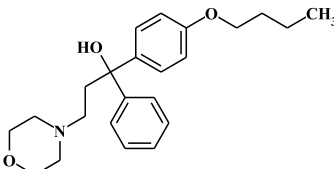
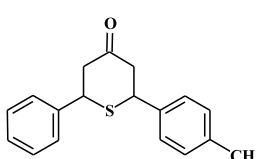
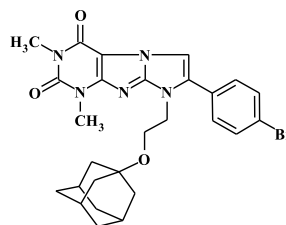


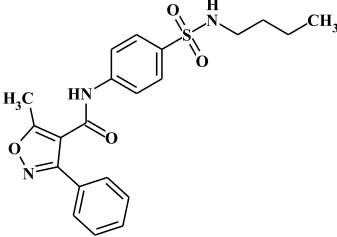
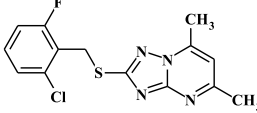
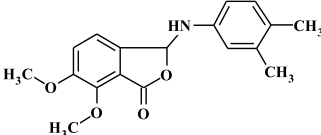
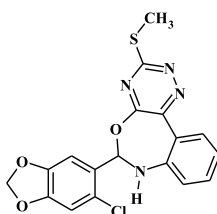
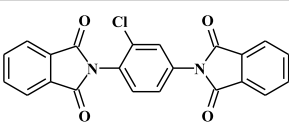
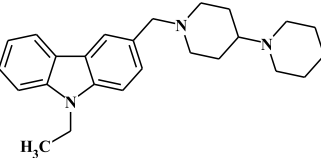
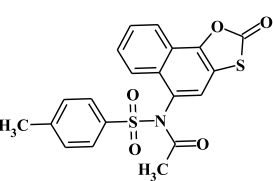
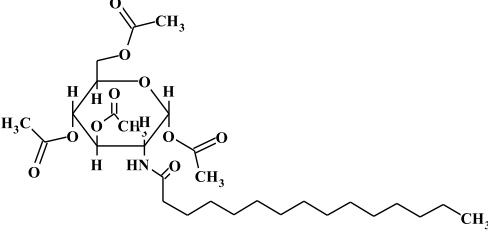
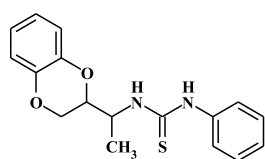
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|--------|--|------|-----|-----|-------|------|---|---|
| 202103 | <br><chem>Cc1ccc(cc1)S(=O)(=O)Nc2ccccc2Nc3cc(Cl)ccc3S</chem><br>$C_{21}H_{16}ClN_3O_3S_2$                 | 0.94 | 74  | N20 | 457.9 | 5.49 | 4 | 2 |
| 201714 | <br><chem>CCOC(=O)Nc1ccc2c(c1)N(C2)C(=O)CN(CC)CC</chem><br>$C_{23}H_{29}N_3O_3$                           | 0.95 | 73  | D12 | 395.5 | 4    | 4 | 1 |
| 215677 | <br><chem>BrC1=CC=C(C=C1)N2C(=O)Nc3ccccc3S2(=O)=O</chem><br>$C_{13}H_9BrN_2O_3S$                          | 0.95 | 113 | J3  | 353.2 | 3.51 | 3 | 1 |
| 209119 | <br><chem>CCN(CC)CCOC(=O)c1cc2c(c1)oc(C)c2C(=O)OCCN(CC)CC</chem><br>$C_{28}H_{40}N_2O_6$                  | 0.97 | 94  | N14 | 500.6 | 4.28 | 4 | 0 |
| 212576 | <br><chem>BrC1=CC=C(C=C1)c2nc3c(nc(=S)s3C(=O)c4cc(OC)c(OC)cc4)C(C)(C)C</chem><br>$C_{20}H_{19}BrN_2O_3S$ | 0.97 | 104 | P19 | 447.3 | 4.75 | 4 | 0 |
| 207580 | <br><chem>O=C1CN2C(=O)N(C2)C(=O)SC1=O</chem><br>$C_{21}H_{22}N_4O_2S_2$                                 | 0.98 | 90  | H3  | 398.5 | 4.15 | 2 | 0 |
| 208567 | <br><chem>CCOC(=O)C(=O)c1ccc2cc3ccccc3cc2c1O</chem><br>$C_{22}H_{16}O_4$                                | 0.98 | 93  | M8  | 344.4 | 4.19 | 2 | 0 |
| 216436 | <br><chem>Nc1ccc(cc1)Oc2ccccc2[N+](=O)[O-]</chem><br>$C_{12}H_{10}N_2O_3$                               | 0.98 | 115 | H17 | 230.2 | 2.58 | 3 | 1 |

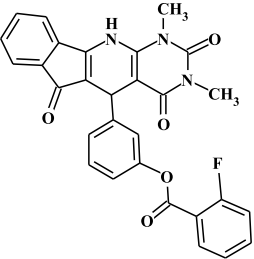
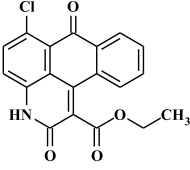
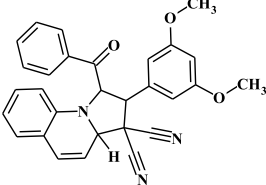
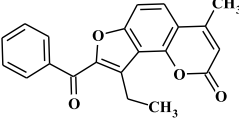
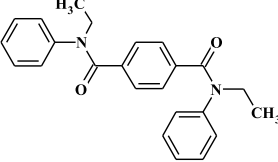
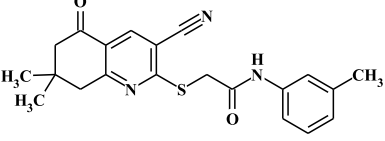
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|--------|---|------------------------------|------|------|-----|-------|------|---|---|
| 200360 |    | <chem>C15H18N2</chem>        | 0.99 | 70   | O1  | 226.3 | 3.1  | 1 | 1 |
| 205261 |    | <chem>C21H22BrClF3N5O</chem> | 1    | 83   | J18 | 532.8 | 4.02 | 4 | 1 |
| 208431 |    | <chem>C19H14N2O</chem>       | 1    | 92   | N18 | 286.3 | 4.81 | 2 | 1 |
| 216542 |    | <chem>C20H25BrN4O3S</chem>   | 1    | 115  | L22 | 481.4 | 2.19 | 5 | 2 |
| 100398 |  | <chem>C23H30ClN3O2S2</chem>  | 1    | 1002 | K11 | 480.1 | 3.22 | 3 | 1 |
| 203313 |  | <chem>C17H21N3O3</chem>      | 1    | 78   | A16 | 315.4 | 0.79 | 3 | 1 |
| 202336 |  | <chem>C18H22N2O2</chem>      | 1    | 75   | P11 | 298.4 | 1.69 | 3 | 3 |
| 203067 |  | <chem>C16H13F3N2OS</chem>    | 1.01 | 77   | F19 | 338.3 | 3.33 | 2 | 1 |

|        |   |      |    |     |       |      |   |   |
|--------|---|------|----|-----|-------|------|---|---|
| 205221 |    | 1.01 | 83 | J8  | 406.5 | 4.81 | 4 | 0 |
|        | <chem>C_{25}H_{30}N_2O_3</chem>   |      |    |     |       |      |   |   |
| 207724 |    | 1.02 | 90 | H18 | 474.5 | 3.27 | 6 | 0 |
|        | <chem>C_{25}H_{18}N_2O_6S</chem>  |      |    |     |       |      |   |   |
| 201213 |    | 1.02 | 72 | I18 | 360.4 | 4.47 | 5 | 1 |
|        | <chem>C_{22}H_{20}N_2O_3</chem>   |      |    |     |       |      |   |   |
| 202806 |   | 1.03 | 76 | L20 | 446.5 | 5.03 | 6 | 0 |
|        | <chem>C_{27}H_{18}N_4O_3</chem>   |      |    |     |       |      |   |   |
| 202886 |  | 1.03 | 77 | K17 | 257.4 | 4.59 | 3 | 1 |
|        | <chem>C_{16}H_{23}N_3</chem>  |      |    |     |       |      |   |   |
| 205970 |  | 1.03 | 85 | D20 | 247.4 | 2.82 | 2 | 1 |
|        | <chem>C_{16}H_{25}NO</chem>   |      |    |     |       |      |   |   |
| 208599 |  | 1.03 | 93 | M16 | 247.4 | 4.19 | 1 | 0 |
|        | <chem>C_{17}H_{20}N</chem>  |      |    |     |       |      |   |   |
| 205234 |  | 1.04 | 83 | D12 | 426.3 | 3.42 | 6 | 0 |
|        | <chem>C_{17}H_{20}BrN_3O_5</chem>   |      |    |     |       |      |   |   |

|               |   |             |           |           |              |             |          |          |
|---------------|---|-------------|-----------|-----------|--------------|-------------|----------|----------|
| 212622        | <br><chem>Cc1cn2c(c1C(=O)NCCCN(CCC)Cc3ccccc3)nc3cc(ccc3S(=O)(=O)N4CCCCC4)C2</chem><br>$C_{32}H_{44}N_4O_4S$                  | 1.04        | 104       | L10       | 580.8        | 4.62        | 6        | 1        |
| 200162        | <br><chem>COc1cc(Br)cc(OC(=O)O)cc1Oc2ccccc2</chem><br>$C_{28}H_{25}BrO_7$  | 1.05        | 69        | C20       | 553.4        | 4.56        | 7        | 1        |
| 202089        | <br><chem>COc1ccc(S)cc1C=C2C(=O)OC(=O)N2c3cc(Cl)cc([N+](=O)[O-])cc3</chem><br>$C_{17}H_{11}ClN_2O_4S$                       | 1.05        | 74        | B18       | 374.8        | 5.01        | 4        | 0        |
| 205295        | <br><chem>c1ccc(cc1)NC2=CN3C=CC=CC=C3N=C2c4ccccc4</chem><br>$C_{20}H_{16}N_2$  | 1.05        | 84        | M3        | 284.4        | 4.7         | 2        | 1        |
| 208839        | <br><chem>CCSC1N(C#N)C(N)C1(C#N)c2ccc(F)cc2</chem><br>$C_{17}H_{17}FN_4S_2$  | 1.05        | 94        | M9        | 360.5        | 3.11        | 4        | 1        |
| <b>209614</b> | <br><chem>Oc1ccc(Cl)cc1C=C2C(=O)N3C=CC=CC=C3N=C2C(=O)c4cc(Cl)cc(Cl)cc4</chem><br>$C_{17}H_{10}Cl_3N_3O_2$                  | <b>1.06</b> | <b>96</b> | <b>K6</b> | <b>394.6</b> | <b>4.58</b> | <b>4</b> | <b>1</b> |
| 205473        | <br><chem>O=C1NC(=O)C2=C1C(=C3C=C(C=C3)C(=O)N4C(=O)C(=C5C=CC(=C5)C=C4N2)C=C6C=CC(=C6)C=C1</chem><br>$C_{20}H_{12}ClN_3O_3$ | 1.07        | 84        | B5        | 377.8        | 2.14        | 4        | 3        |

|        |   |      |    |     |       |      |   |   |
|--------|---|------|----|-----|-------|------|---|---|
| 208677 | <br><chem>Cc1c2c(c3ccccc3n1C)nc(=O)n2c4ccccc4S4</chem>   | 1.07 | 93 | J13 | 387.4 | 1.13 | 5 | 0 |
| 200130 | <br><chem>CCOC(=O)NCCc1ccc(OC)c(OC)c1N2CCCN(C2)S(=O)(=O)c3cc4nn[nH]4cc3</chem>   | 1.08 | 69 | C12 | 518.6 | 3.07 | 7 | 1 |
| 205563 | <br><chem>COCCOc1ccc(cc1)C(O)CN2CCOCC2</chem>  | 1.08 | 84 | F6  | 369.5 | 3.8  | 4 | 1 |
| 205343 | <br><chem>CC1=CC=C(C=C1)C2=CC(=CC=C2)SC(=O)C2=CC=CC=C2</chem>  | 1.09 | 84 | M15 | 282.4 | 4.87 | 1 | 0 |
| 202554 | <br><chem>BrC1=CC=C(C=C1)N2C3C4C5C6C7C8C9C10C11C12C13C14C15C16C17C18C19C20C21C22C23C24C25C26C27C28C29C30C31C32C33C34C35C36C37C38C39C40C41C42C43C44C45C46C47C48C49C50C51C52C53C54C55C56C57C58C59C60C61C62C63C64C65C66C67C68C69C70C71C72C73C74C75C76C77C78C79C80C81C82C83C84C85C86C87C88C89C90C91C92C93C94C95C96C97C98C99C100C101C102C103C104C105C106C107C108C109C110C111C112C113C114C115C116C117C118C119C120C121C122C123C124C125C126C127C128C129C130C131C132C133C134C135C136C137C138C139C140C141C142C143C144C145C146C147C148C149C150C151C152C153C154C155C156C157C158C159C160C161C162C163C164C165C166C167C168C169C170C171C172C173C174C175C176C177C178C179C180C181C182C183C184C185C186C187C188C189C190C191C192C193C194C195C196C197C198C199C200C201C202C203C204C205C206C207C208C209C210C211C212C213C214C215C216C217C218C219C220C221C222C223C224C225C226C227C228C229C230C231C232C233C234C235C236C237C238C239C240C241C242C243C244C245C246C247C248C249C250C251C252C253C254C255C256C257C258C259C260C261C262C263C264C265C266C267C268C269C270C271C272C273C274C275C276C277C278C279C280C281C282C283C284C285C286C287C288C289C290C291C292C293C294C295C296C297C298C299C300C301C302C303C304C305C306C307C308C309C310C311C312C313C314C315C316C317C318C319C320C321C322C323C324C325C326C327C328C329C330C331C332C333C334C335C336C337C338C339C340C341C342C343C344C345C346C347C348C349C350C351C352C353C354C355C356C357C358C359C360C361C362C363C364C365C366C367C368C369C370C371C372C373C374C375C376C377C378C379C380C381C382C383C384C385C386C387C388C389C390C391C392C393C394C395C396C397C398C399C400C401C402C403C404C405C406C407C408C409C410C411C412C413C414C415C416C417C418C419C420C421C422C423C424C425C426C427C428C429C430C431C432C433C434C435C436C437C438C439C440C441C442C443C444C445C446C447C448C449C450C451C452C453C454C455C456C457C458C459C460C461C462C463C464C465C466C467C468C469C470C471C472C473C474C475C476C477C478C479C480C481C482C483C484C485C486C487C488C489C490C491C492C493C494C495C496C497C498C499C500C501C502C503C504C505C506C507C508C509C510C511C512C513C514C515C516C517C518C519C520C521C522C523C524C525C526C527C528C529C530C531C532C533C534C535C536C537C538C539C540C541C542C543C544C545C546C547C548C549C550C551C552C553C554C555C556C557C558C559C560C561C562C563C564C565C566C567C568C569C570C571C572C573C574C575C576C577C578C579C580C581C582C583C584C585C586C587C588C589C590C591C592C593C594C595C596C597C598C599C600C601C602C603C604C605C606C607C608C609C610C611C612C613C614C615C616C617C618C619C620C621C622C623C624C625C626C627C628C629C630C631C632C633C634C635C636C637C638C639C640C641C642C643C644C645C646C647C648C649C650C651C652C653C654C655C656C657C658C659C660C661C662C663C664C665C666C667C668C669C670C671C672C673C674C675C676C677C678C679C680C681C682C683C684C685C686C687C688C689C690C691C692C693C694C695C696C697C698C699C700C701C702C703C704C705C706C707C708C709C710C711C712C713C714C715C716C717C718C719C720C721C722C723C724C725C726C727C728C729C730C731C732C733C734C735C736C737C738C739C740C741C742C743C744C745C746C747C748C749C750C751C752C753C754C755C756C757C758C759C760C761C762C763C764C765C766C767C768C769C770C771C772C773C774C775C776C777C778C779C780C781C782C783C784C785C786C787C788C789C790C791C792C793C794C795C796C797C798C799C800C801C802C803C804C805C806C807C808C809C810C811C812C813C814C815C816C817C818C819C820C821C822C823C824C825C826C827C828C829C830C831C832C833C834C835C836C837C838C839C840C841C842C843C844C845C846C847C848C849C850C851C852C853C854C855C856C857C858C859C860C861C862C863C864C865C866C867C868C869C870C871C872C873C874C875C876C877C878C879C880C881C882C883C884C885C886C887C888C889C890C891C892C893C894C895C896C897C898C899C900C901C902C903C904C905C906C907C908C909C910C911C912C913C914C915C916C917C918C919C920C921C922C923C924C925C926C927C928C929C930C931C932C933C934C935C936C937C938C939C940C941C942C943C944C945C946C947C948C949C950C951C952C953C954C955C956C957C958C959C960C961C962C963C964C965C966C967C968C969C970C971C972C973C974C975C976C977C978C979C980C981C982C983C984C985C986C987C988C989C990C991C992C993C994C995C996C997C998C999C1000C1001C1002C1003C1004C1005C1006C1007C1008C1009C1010C1011C1012C1013C1014C1015C1016C1017C1018C1019C1020C1021C1022C1023C1024C1025C1026C1027C1028C1029C1030C1031C1032C1033C1034C1035C1036C1037C1038C1039C1040C1041C1042C1043C1044C1045C1046C1047C1048C1049C1050C1051C1052C1053C1054C1055C1056C1057C1058C1059C1060C1061C1062C1063C1064C1065C1066C1067C1068C1069C1070C1071C1072C1073C1074C1075C1076C1077C1078C1079C1080C1081C1082C1083C1084C1085C1086C1087C1088C1089C1090C1091C1092C1093C1094C1095C1096C1097C1098C1099C1100C1101C1102C1103C1104C1105C1106C1107C1108C1109C1110C1111C1112C1113C1114C1115C1116C1117C1118C1119C1120C1121C1122C1123C1124C1125C1126C1127C1128C1129C1130C1131C1132C1133C1134C1135C1136C1137C1138C1139C1140C1141C1142C1143C1144C1145C1146C1147C1148C1149C1150C1151C1152C1153C1154C1155C1156C1157C1158C1159C1160C1161C1162C1163C1164C1165C1166C1167C1168C1169C1170C1171C1172C1173C1174C1175C1176C1177C1178C1179C1180C1181C1182C1183C1184C1185C1186C1187C1188C1189C1190C1191C1192C1193C1194C1195C1196C1197C1198C1199C1200C1201C1202C1203C1204C1205C1206C1207C1208C1209C1210C1211C1212C1213C1214C1215C1216C1217C1218C1219C1220C1221C1222C1223C1224C1225C1226C1227C1228C1229C1230C1231C1232C1233C1234C1235C1236C1237C1238C1239C1240C1241C1242C1243C1244C1245C1246C1247C1248C1249C1250C1251C1252C1253C1254C1255C1256C1257C1258C1259C1260C1261C1262C1263C1264C1265C1266C1267C1268C1269C1270C1271C1272C1273C1274C1275C1276C1277C1278C1279C1280C1281C1282C1283C1284C1285C1286C1287C1288C1289C1290C1291C1292C1293C1294C1295C1296C1297C1298C1299C1300C1301C1302C1303C1304C1305C1306C1307C1308C1309C1310C1311C1312C1313C1314C1315C1316C1317C1318C1319C1320C1321C1322C1323C1324C1325C1326C1327C1328C1329C1330C1331C1332C1333C1334C1335C1336C1337C1338C1339C1340C1341C1342C1343C1344C1345C1346C1347C1348C1349C1350C1351C1352C1353C1354C1355C1356C1357C1358C1359C1360C1361C1362C1363C1364C1365C1366C1367C1368C1369C1370C1371C1372C1373C1374C1375C1376C1377C1378C1379C1380C1381C1382C1383C1384C1385C1386C1387C1388C1389C1390C1391C1392C1393C1394C1395C1396C1397C1398C1399C1400C1401C1402C1403C1404C1405C1406C1407C1408C1409C1410C1411C1412C1413C1414C1415C1416C1417C1418C1419C1420C1421C1422C1423C1424C1425C1426C1427C1428C1429C1430C1431C1432C1433C1434C1435C1436C1437C1438C1439C1440C1441C1442C1443C1444C1445C1446C1447C1448C1449C1450C1451C1452C1453C1454C1455C1456C1457C1458C1459C1460C1461C1462C1463C1464C1465C1466C1467C1468C1469C1470C1471C1472C1473C1474C1475C1476C1477C1478C1479C1480C1481C1482C1483C1484C1485C1486C1487C1488C1489C1490C1491C1492C1493C1494C1495C1496C1497C1498C1499C1500C1501C1502C1503C1504C1505C1506C1507C1508C1509C1510C1511C1512C1513C1514C1515C1516C1517C1518C1519C1520C1521C1522C1523C1524C1525C1526C1527C1528C1529C1530C1531C1532C1533C1534C1535C1536C1537C1538C1539C1540C1541C1542C1543C1544C1545C1546C1547C1548C1549C1550C1551C1552C1553C1554C1555C1556C1557C1558C1559C1560C1561C1562C1563C1564C1565C1566C1567C1568C1569C1570C1571C1572C1573C1574C1575C1576C1577C1578C1579C1580C1581C1582C1583C1584C1585C1586C1587C1588C1589C1590C1591C1592C1593C1594C1595C1596C1597C1598C1599C1600C1601C1602C1603C1604C1605C1606C1607C1608C1609C1610C1611C1612C1613C1614C1615C1616C1617C1618C1619C1620C1621C1622C1623C1624C1625C1626C1627C1628C1629C1630C1631C1632C1633C1634C1635C1636C1637C1638C1639C1640C1641C1642C1643C1644C1645C1646C1647C1648C1649C1650C1651C1652C1653C1654C1655C1656C1657C1658C1659C1660C1661C1662C1663C1664C1665C1666C1667C1668C1669C1670C1671C1672C1673C1674C1675C1676C1677C1678C1679C1680C1681C1682C1683C1684C1685C1686C1687C1688C1689C1690C1691C1692C1693C1694C1695C1696C1697C1698C1699C1700C1701C1702C1703C1704C1705C1706C1707C1708C1709C1710C1711C1712C1713C1714C1715C1716C1717C1718C1719C1720C1721C1722C1723C1724C1725C1726C1727C1728C1729C1730C1731C1732C1733C1734C1735C1736C1737C1738C1739C1740C1741C1742C1743C1744C1745C1746C1747C1748C1749C1750C1751C1752C1753C1754C1755C1756C1757C1758C1759C1760C1761C1762C1763C1764C1765C1766C1767C1768C1769C1770C1771C1772C1773C1774C1775C1776C1777C1778C1779C1780C1781C1782C1783C1784C1785C1786C1787C1788C1789C1790C1791C1792C1793C1794C1795C1796C1797C1798C1799C1800C1801C1802C1803C1804C1805C1806C1807C1808C1809C1810C1811C1812C1813C1814C1815C1816C1817C1818C1819C1820C1821C1822C1823C1824C1825C1826C1827C1828C1829C1830C1831C1832C1833C1834C1835C1836C1837C1838C1839C1840C1841C1842C1843C1844C1845C1846C1847C1848C1849C1850C1851C1852C1853C1854C1855C1856C1857C1858C1859C1860C1861C1862C1863C1864C1865C1866C1867C1868C1869C1870C1871C1872C1873C1874C1875C1876C1877C1878C1879C1880C1881C1882C1883C1884C1885C1886C1887C1888C1889C1890C1891C1892C1893C1894C1895C1896C1897C1898C1899C1900C1901C1902C1903C1904C1905C1906C1907C1908C1909C1910C1911C1912C1913C1914C1915C1916C1917C1918C1919C1920C1921C1922C1923C1924C1925C1926C1927C1928C1929C1930C1931C1932C1933C1934C1935C1936C1937C1938C1939C1940C1941C1942C1943C1944C1945C1946C1947C1948C1949C1950C1951C1952C1953C1954C1955C1956C1957C1958C1959C1960C1961C1962C1963C1964C1965C1966C1967C1968C1969C1970C1971C1972C1973C1974C1975C1976C1977C1978C1979C1980C1981C1982C1983C1984C1985C1986C1987C1988C1989C1990C1991C1992C1993C1994C1995C1996C1997C1998C1999C2000C2001C2002C2003C2004C2005C2006C2007C2008C2009C2010C2011C2012C2013C2014C2015C2016C2017C2018C2019C2020C2021C2022C2023C2024C2025C2026C2027C2028C2029C2030C2031C2032C2033C2034C2035C2036C2037C2038C2039C2040C2041C2042C2043C2044C2045C2046C2047C2048C2049C2050C2051C2052C2053C2054C2055C2056C2057C2058C2059C2060C2061C2062C2063C2064C2065C2066C2067C2068C2069C2070C2071C2072C2073C2074C2075C2076C2077C2078C2079C2080C2081C2082C2083C2084C2085C2086C2087C2088C2089C2090C2091C2092C2093C2094C2095C2096C2097C2098C2099C2100C2101C2102C2103C2104C2105C2106C2107C2108C2109C2110C2111C2112C2113C2114C2115C2116C2117C2118C2119C2120C2121C2122C2123C2124C2125C2126C2127C2128C2129C2130C2131C2132C2133C2134C2135C2136C2137C2138C2139C2140C2141C2142C2143C2144C2145C2146C2147C2148C2149C2150C2151C2152C2153C2154C2155C2156C2157C2158C2159C2160C2161C2162C2163C2164C2165C2166C2167C2168C2169C2170C2171C2172C2173C2174C2175C2176C2177C2178C2179C2180C2181C2182C2183C2184C2185C2186C2187C2188C2189C2190C2191C2192C2193C2194C2195C2196C2197C2198C2199C2200C2201C2202C2203C2204C2205C2206C2207C2208C2209C2210C2211C2212C2213C2214C2215C2216C2217C2218C2219C2220C2221C2222C2223C2224C2225C2226C2227C2228C2229C2230C2231C2232C2233C2234C2235C2236C2237C2238C2239C2240C2241C2242C2243C2244C2245C2246C2247C2248C2249C2250C2251C2252C2253C2254C2255C2256C2257C2258C2259C2260C2261C2262C2263C2264C2265C2266C2267C2268C2269C2270C2271C2272C2273C2274C2275C2276C2277C2278C2279C2280C2281C2282C2283C2284C2285C2286C2287C2288C2289C2290C2291C2292C2293C2294C2295C2296C2297C2298C2299C2300C2301C2302C2303C2304C2305C2306C2307C2308C2309C2310C2311C2312C2313C2314C2315C2316C2317C2318C2319C2320C2321C2322C2323C2324C2325C2326C2327C2328C2329C2330C2331C2332C2333C2334C2335C2336C2337C2338C2339C2340C2341C2342C2343C2344C2345C2346C2347C2348C2349C2350C2351C2352C2353C2354C2355C2356C2357C2358C2359C2360C2361C2362C2363C2364C2365C2366C2367C2368C2369C2370C2371C2372C2373C2374C2375C2376C2377C2378C2379C2380C2381C2382C2383C2384C2385C2386C2387C2388C2389C2390C2391C2392C2393C2394C2395C2396C2397C2398C2399C2400C2401C2402C2403C2404C2405C2406C2407C2408C2409C2410C2411C2412C2413C2414C2415C2416C2417C2418C2419C2420C2421C2422C2423C2424C2425C2426C2427C2428C2429C2430C2431C2432C2433C2434C2435C2436C2437C2438C2439C2440C2441C2442C2443C2444C2445C2446C2447C2448C2449C2450C2451C2452C2453C2454C2455C2456C2457C2458C2459C2460C2461C2462C2463C2464C2465C2466C2467C2468C2469C2470C2471C2472C2473C2474C2475C2476C2477C2478C2479C2480C2481C2482C2483C2484C2485C2486C2487C2488C2489C2490C2491C2492C2493C2494C2495C2496C2497C2498C2499C2500C2501C2502C2503C2504C2505C2506C2507C2508C2509C2510C2511C2512C2513C2514C2515C2516C2517C2518C2519C2520C2521C2522C2523C2524C2525C2526C2527C2528C2529C2530C2531C2532C2533C2534C2535C2536C2537C2538C2539C2540C2541C2542C2</chem> |      |    |     |       |      |   |   |

|        |   |      |    |     |       |      |   |   |
|--------|---|------|----|-----|-------|------|---|---|
| 205425 | <br><chem>CCCCS(=O)(=O)Nc1ccc(cc1)NC(=O)c2cc3ccccc3on2C</chem>   | 1.13 | 84 | A16 | 413.5 | 3.82 | 4 | 2 |
| 202977 | <br><chem>Cc1cc(C)nnc1SCc2cc(F)cc(Cl)c2</chem>   | 1.15 | 77 | A20 | 322.8 | 4.22 | 4 | 0 |
| 204332 | <br><chem>COc1cc(OC)c2c(c1)oc(=O)c2NCc3cc(C)cc(C)c3</chem>   | 1.16 | 81 | G6  | 313.3 | 3.84 | 4 | 1 |
| 202747 | <br><chem>CSc1nn2c(c1)oc3c4cc(Cl)cc5ccccc4n3c5</chem>  | 1.17 | 76 | F6  | 400.8 | 4.43 | 7 | 1 |
| 201913 | <br><chem>O=C1c2ccccc2N1c3ccc(Cl)cc3N4C(=O)c5ccccc5C4=O</chem>  | 1.18 | 74 | A18 | 402.8 | 3.78 | 4 | 0 |
| 208469 | <br><chem>CN1c2cc3ccccc3c2CN1Cc4ccccc4N5CCCCC5</chem>  | 1.18 | 93 | I5  | 375.5 | 4.49 | 2 | 0 |
| 209044 | <br><chem>Cc1ccc(cc1)S(=O)(=O)N2C(=O)OC2c3cc4ccccc4s3</chem>   | 1.18 | 94 | H17 | 413.5 | 4.34 | 5 | 0 |
| 201996 | <br><chem>CCCCCCCCCCCCCCCCCOCC(=O)N[C@@H]1[C@H](OC(=O)C)[C@H](OC(=O)C)[C@H](OC(=O)C)[C@H]1OC(=O)C</chem> | 1.19 | 74 | H15 | 571.7 | 4.58 | 6 | 1 |
| 205599 | <br><chem>CC(C)C1OC2c3ccccc3OC2OC1NC(=S)Nc4ccccc4</chem>   | 1.19 | 84 | N14 | 314.4 | 3.78 | 2 | 2 |

|        |   |                                  |      |    |     |       |      |   |   |
|--------|---|----------------------------------|------|----|-----|-------|------|---|---|
| 205464 |    | <chem>C_{29}H_{20}FN_3O_5</chem> | 1.19 | 84 | P1  | 509.5 | 3.78 | 5 | 1 |
| 202563 |    | <chem>C_{19}H_{12}ClNO_4</chem>  | 1.2  | 76 | E4  | 353.8 | 3.44 | 3 | 1 |
| 202803 |    | <chem>C_{29}H_{23}N_3O_3</chem>  | 1.2  | 76 | F20 | 461.5 | 4.78 | 6 | 0 |
| 202733 |    | <chem>C_{21}H_{16}O_4</chem>     | 1.2  | 76 | J2  | 332.3 | 4.58 | 2 | 0 |
| 203442 |  | <chem>C_{24}H_{24}N_2O_2</chem>  | 1.2  | 78 | D4  | 372.5 | 4.6  | 2 | 0 |
| 205444 |  | <chem>C_{21}H_{21}N_3O_2S</chem> | 1.2  | 84 | G20 | 379.5 | 3.72 | 4 | 1 |

**Suppl. Tab. 1.** Primary screening of 17,700 small molecules identified 83 inhibitors of the cAMP-induced redistribution of AQP2 in MCD4 cells. Compounds are ranked according to increasing ratios of plasma membrane/intracellular AQP2 fluorescence signal intensities. Compounds with ratios  $\leq 1.2$  were defined as inhibitory (a predominant intracellular localization of AQP2). Secondary screening revealed that 17 of the hits (*italic*) inhibited the AQP2 redistribution in MCD4 cells concentration-dependently. The inhibitory effect of 5 compounds (*italic and bold*) was also observed in IMCD cells. Compound ID numbers (ID), compound library positions (plate, well), molecular weight (MW), partition coefficient (logP), numbers of proton donor (H-Don.) and acceptor (H-Acc.) moieties are given for each substance.