Supporting Information

Suppoting information

Characterization of quinoline yellow dyes as transient aryl hydrocarbon receptor agonists

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Content

Figure S1: AHR activation of a polystyrene DMSO migrate in the XEER assay. Cells were stimulated with the DMSO migrate of a yellow polystyrene cell scraper, diluted 1:1000 in HEPES buffered HF-DMEM containing D-luciferin. Total and filtered luminescence was recorded hourly and ELuc luminescence was calculated as described in the methods section. TCDD (10 nM) was used as positive control, 0.1% DMSO as negative control. Data represent the mean ± SEM of one representative experiment performed in six replicates.

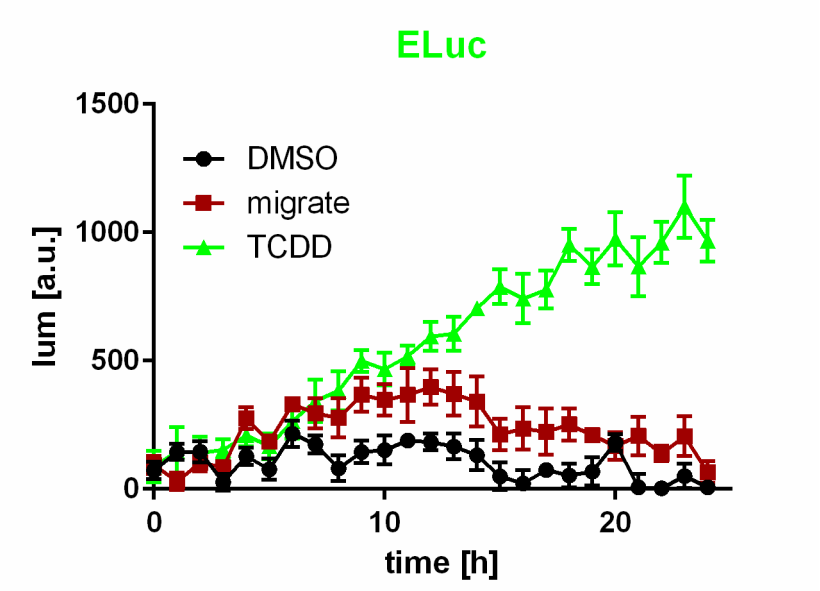


Figure S2: AHR and AHR regulated CYP protein expression in MCF7 and HepG2 cells. Cells were exposed to 10 nM TCDD or to 0.1 % DMSO for 24 h. Protein was isolated and equal amounts were separated on an SDS-PAGE and blotted onto a nitrocellulose membrane. Blots were hybridized with respective primary antibodies and secondary antibodies conjugated to a peroxidase. Membranes were then re-probed with an antibody against tubulin as loading control.

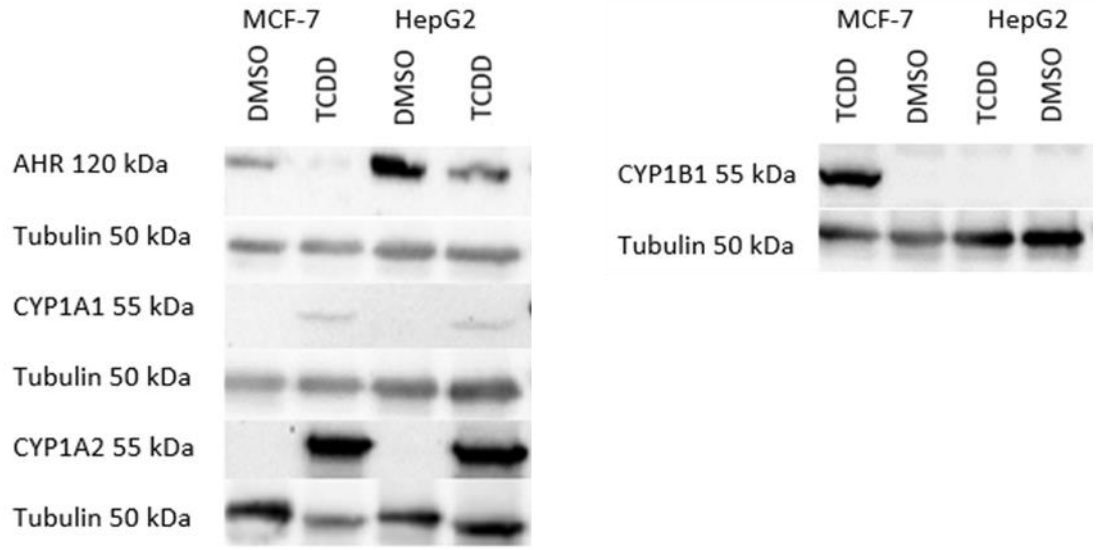


Figure S3 Inhibition of basal estrogen receptor activity by LY. . SLR luminescence recordings of XEER cells stimulated with 17β-estradiol (E2, 1 nM) or LY as indicated, using DMSO (0.1 %, v/v) as negative control. Shown is the mean ± SEM of one representative experiment performed in six replicates.

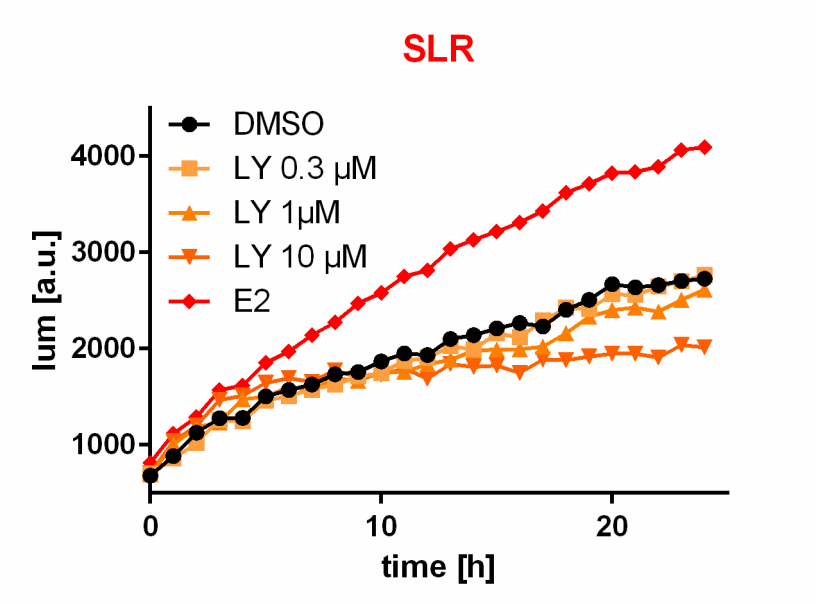
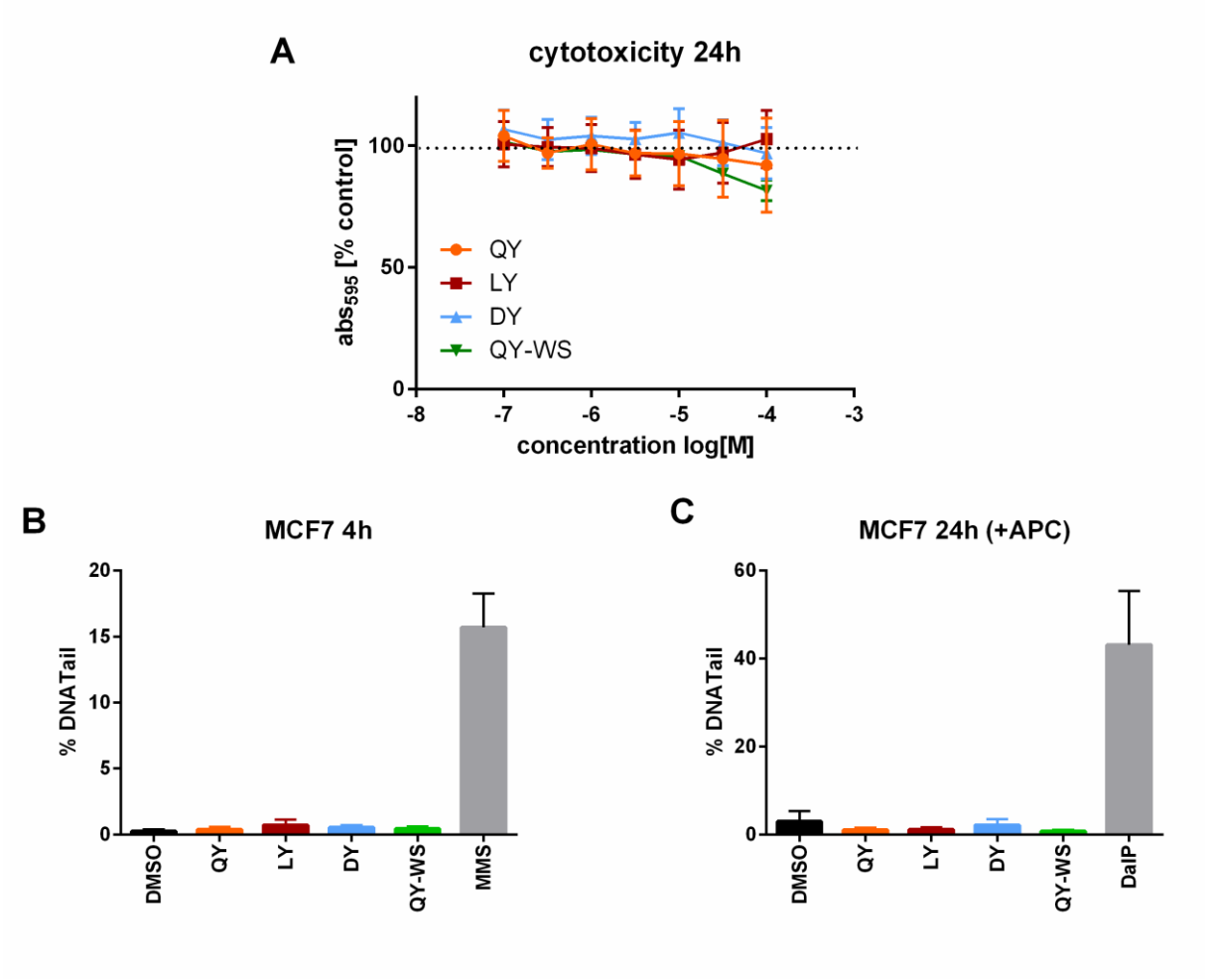


Figure S4 Comet assay with 100 µM dyes in MCF7 cells. Cytotoxicity was tested using the MTT assay after 24 h of exposure to the dyes in the indicated concentrations (A). Data represent the mean ± SEM from three independent experiments, each performed in triplicates and normalized to the solvent control (1 % v/v DMSO). MCF7 cells were exposed to QY, LY, DY or QY-WS (100 µM) as indicated (B and C). Methyl methanesulfonate (MMS, 40 µM) and dibenzo[*a,l*]pyrene (DalP, 0.1 µM) were used as positive controls for the 4 h standard assay (MMS in panel B) and the 24 h APC-variant (DalP in panel C), respectively. DMSO (1 % v/v) was used as negative control. Percentage of DNA forming the tail was determined for at least 50 individual cells per slide with two slides per treatment. For each slide the median of tail DNA percentage was determined. Data represent the mean ± SEM from the medians of three independent experiments.



Additional Methods:

Cytotoxicity assay

MCF7 cells (2 x 10^4 cells/100 µl) were seeded in 96-well plates and exposed to the dyes (0 – 100 µM). The final concentration of DMSO did not exceed 1 % (v/v). After 24 h exposure, 50 µl of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg/mL medium) were added and incubated for 1 - 2 h at 37°C, 5% CO_2. Afterwards, formazan crystals were dissolved by adding 100 µL DMSO into each well. The absorption of the samples was measured in triplicates on a microplate reader at a wavelength 595 nm.