Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Identification of synthetic inhibitors for the DNA binding of intrinsically disordered circadian clock transcription factors

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2) General procedures

General procedures of biological experiments

All reagents used in biological experiments were purchased from commercial suppliers (Sigma-Aldrich, Wako Pure Chemical Industries, Nacalai tesque) and used without further purification unless otherwise stated. All optical density measured by the absorption at 600 nm (OD_{600}) and protein concentration was measured by Thermo (NANODROP ONE) apparatus.

Analytical HPLC

HPLC were recorded on a JASCO (EXTREMA) apparatus consisting of prominence UV detector (UV-4075). ODS column (4.6 x 150, Nacalai tesque, COSMSIL 38144-31) was used with UV detection at 220 nm and 254 nm, a flow rate of 1.0 mL/min. (10-90% of MeOH/0.1%TFA in H_2O) for 20 min.

Preparation of sample for SDS-PAGE and analysis by SDS-PAGE

After protein sample solution (13.5 μL) was added in a microtube, 2x sample buffer solution without 2-ME for SDS-PAGE (15 μL, nacalai tesque) and 2-mercaptoethanol (1.5 μL, Wako) were added. The mixture was mixed by vortex, and heated at 95 °C for 3 min. Each sample (10 μL) was added on a well of 12% acrylamide SDS-PAGE gel. Electrophoresis was completed at 60 mA for 45 minutes to 1 hour on BIO-RAD (Power Pac basic power supply) apparatus. Imaging of SDS-PAGE gel was performed by BIO-RAD (Gel Doc EZ Imager) apparatus.

3) Methods for Biochemical/Biological Experiment

a) Expression and purification of recombinant mus musculus BMAL1 and CLOCK proteins

i) Construction of plasmid DNA

The DNA sequence encoding mouse BMAL1 (1-448) or CLOCK (1-383) was amplified from mBMAL1 cDNA or mCLOCK cDNA (kindly provided by Dr. H. Tei), respectively, by PCR using following primers (5′-3′); BMAL1 fwd (cgcggatccg GCA GAC CAG AGA ATG GAC), BMAL1 (448) rev (ttctcgag tca GCC TTC CAG GAC ATT GGC); CLOCK fwd (cgcggatccg GTG TTT ACC GTA AGC TG), CLOCK (383) rev (ttctcgag tca AGC CCT AAC TTC TGC ATA ACT). Amplified DNA fragments were digested by BamHI and XhoI and inserted between BamHI and XhoI sites of pET32b (Novagen), which contained the TrxA and His6-tag-encoding sequences.

ii) Protein expression and purification

The plasmid (48.2 ng / μL, 0.2 μL) was added to *Escherichia coli* strain BL21 (DE3) cells (10 μL), and the solution was mixed by gentle tapping. The mixture was incubated at 42 °C for 30 sec, and the solution was placed on ice and left for 1 min. LB medium (30 μL) was added into the mixture. The cells were placed on LB agar plate (ampicillin 100 μg / mL), and the solution was spread by an inoculating needle. After incubation of the plate at 37 °C for 9 h, a single colony was picked up and inoculated into LB media (5 mL) and incubated at 37 °C for overnight (190 rpm) by TAITEC (BR-43FL) incubator. The mini culture (4.5 mL) was transferred into LB media (1L) and further incubated until OD_{600} reached to 0.8 - 1.0. The large culture solution was cooled on ice for 15 min, and then isopropyl β-D-1-thiogalactopyranoside (IPTG, 1 M, 1 mL) was added (final concentration of IPTG = 1 mM) , on the culture and gently stirred. After incubation of the mixture at 37 °C for 1 h (190 rpm), the cells were collected by centrifugation at 4 °C for 5 min (5,000 g).

The resulting pellet (~2 g) was suspended in lysis buffer (NP-40 1%, 10 mM Tris, 150 mM NaCl, protease inhibitor 100×, pH 8.0), and was disrupted on ice using MISONIX XL2000 (sonic time = 10 sec., rest time = 30 sec., sonic total time = 5 min., power = 10). The mixture was centrifuged at 4 °C for 20 min (15,000 g), and the resulting pellet was obtained by removing supernatant by decantation. The resulting pellet was dissolved in urea buffer (8 M urea, 10 mM Tris, 150 mM NaCl, pH 8.0, 5 mL) and centrifuged at 6 °C for 40 min (15,000 g), and the supernatant was transferred into a tube (50 mL). This step was repeated twice to remove cellular debris completely. The supernatant fraction was applied to a Ni-NTA column, and additional buffer (8 M urea, 10 mM Tris, 150 mM NaCl, 10 mM imidazole, pH 8.0, 7 mL) was added, and incubated at 4 °C under rotation for 40 min – 1 h to let the proteins bound to the resin. After removing pass through, the column was subsequently washed with with wash buffer (8 M urea, 10 mM Tris, 150 mM NaCl, 10 mM imidazole, pH 8.0, 40 mL), and the protein was eluted with increasing concentration of imidazole (50 to 250 mM). The fractions containing protein were collected, and transferred into two dialysis membrane tubes (MWCO 14 kDa, approximately 40 mL per a tube). The tubes were combined and placed into a 1L-beaker, and then the consecutive dialysis was first performed against dialysis buffer (400 mL, 10 mM Tris, 150 mM NaCl, 10 %v/v glycerol, 1 mM DTT, pH 8.0) at 4 °C for 2 h, the buffer was changed (400 mL) and stood for 2h, the buffer was changed (500 ml) and stood for 2h, the buffer was changed (800 ml) and stood for 2h. This step was repeated twice. As a result, the final urea concentration was reduced to 300 – 600 μM. The dialyzed protein solution was concentrated using VIVA SPIN 15 (MWCO: 30 kDa), at 4 °C (3,800 g, 30 min). This step was repeated for – 10 times until the concentration reached more than 1.5 mg / mL. As a result, ~ 1 mL of protein solution (1.5 mg / mL) was obtained, which were stored as a 10 μL aliquot at -80 °C.

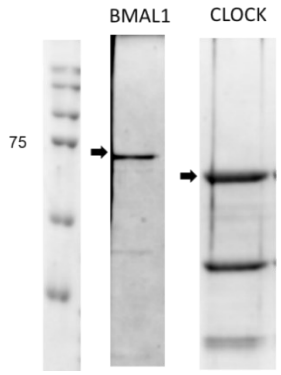


Figure S1. The results of SDS-PAGE analysis of BMAL1 (Calcd. Mw = 68239) and CLOCK (Calcd. Mw = 62104) after purification. The gels were stained by Coomassie Brilliant Blue.

b) Expression and purification of Sp1ZF protein

i) Construction of plasmid DNA

The DNA sequence encoding Sp1(530-623) fused with His6-tag at the C-terminus of Sp1(530-623) was amplified from Sp1(530-623)/pEV3b using T7 promoter primer and His-tag-encoding reverse primer. The DNA fragment was digested by NdeI and XhoI followed by insertion between NdeI and XhoI sites of pET17b (Novagen).

ii) Protein expression and purification

E coli strain BL21(DE3) cells were transformed with Sp1ZF/pET17b. A single colony was picked into liquid LB medium and incubated at 37°C until the OD_{600} reached 0.6. Then 0.1 mM IPTG and 0.1 mM $ZnCl_2$ were added and the *E. coli* cells were cultured overnight at 20°C. The bacterial cells were lysed by sonication in His-binding buffer (20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4). The lysates were centrifuged, and the supernatants were loaded on Ni-NTA HisTrap FF column (GE Healthcare). After washing using His-washing buffer (20 mM NaH_2PO_4, 500 mM NaCl, 32.5 mM imidazole, pH 7.4, the Sp1ZF protein with His6-tag was eluted using His-elution buffer (20 mM NaH_2PO_4, 500 mM NaCl, 250 mM imidazole, pH 7.4). Fractions were concentrated by ultrafiltration using an AmiconUltra 0.5mL filter device (10 KDa NMWL) and transferred to stock buffer (25 mM Tris-HCl (pH 7.5), 100 mM NaCl).

c) Fluorescence polarization assay for binding of BMAL1 / CLOCK to an E-box DNA fragment

i) Fluorescence polarization binding assay

The complementary oligonucleotides (Invitrogen), upper (5'- GCGCGGTCACGTTTTCCACT -3') and lower (5'- FAM - AGTGGAAAACGTGACCGCGC -3'), were annealed and used for the probe Per 2 E-box DNA. And the complementary oligonucleotides (Invitrogen), upper (5'-

GCGCGGTGCTAGTTTCCACT -3') and lower (5'- Fluorescein - AGTGGAAACTAGCACCG CGC -3'), were annealed and used for the probe Per 2 mutant E -box DNA.

A droplet of compound solution (5 mM by DMSO, 1.0 μL) and another droplet of protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, 86.7 μL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 μL), the mixture (total volume 100 μL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes.The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm).

ii) Fluorescence polarization titration assay

A series of dilution of the protein solutions (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM) was first carried out with buffer solution (10 mM Tris, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0) as follows: To a well buffer solution (20 μL) was placed. To the well was added protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL), respectively. After mixing the solution by pipetting, the mixture (10 μL) was transferred into the next well containing buffer solution (20 μL), and mixed by pipetting. This step was repeated for 6 times to fill the 8 consecutive wells with the series diluted protein solutions. To each well, additional buffer solution (70 μL) and DNA solution (100 nM, 10 μL) were added. After shaking the plate (each well contains total volume of 100 μL solution) at 25 °C for 1 min (900 rpm), the mixture was gently incubated on a rocker at 25 °C for 19 minutes. The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm, n=3).

iii) Analysis of PEG200 effect to change fluorescence polarization intensity

A droplet of compound solution (5 mM by DMSO, 1.0 μL) and another droplet of protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, or 10 mM Tris HCl, 150 mM NaCl, pH 8.0, 86.7 μL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 μL), the mixture (total volume 100 μL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes. The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm).

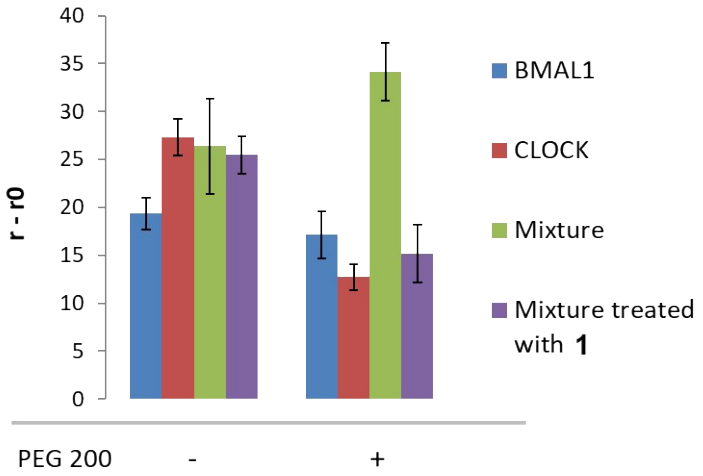


Figure S2. Effect of PEG200 (10% v/v) on the binding of BMAL1 / CLOCK to E-box DNA. n=3.

d) Compound library screening

Libraries of heterocycles (1785 compounds, 5 mM, Tripos Receptor Research) was used in this study. A droplet of compound solution (5 mM by DMSO, 1.0 μL) and another droplet of protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, or 10 mM Tris HCl, 150 mM NaCl, pH 8.0, 86.7 μL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 μL), the mixture (total volume 100 μL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes. The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm).

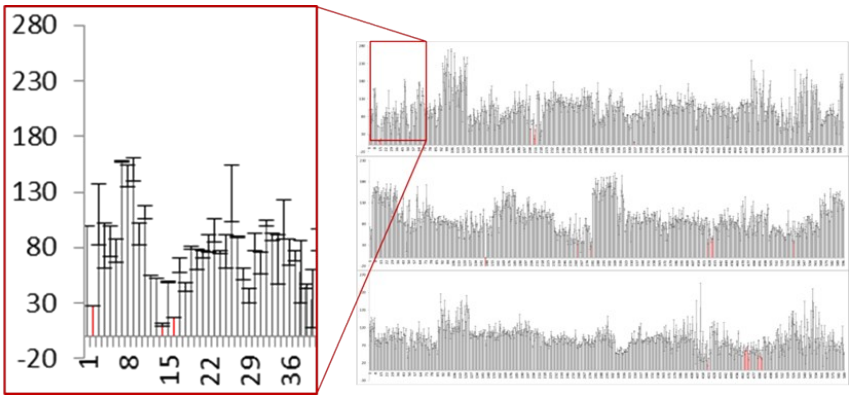


Figure S3. The results of the screening of compound libraries. 27 compounds were found as positive. Tukey-Kramer test was used to compare differences between groups, and results were considered statistically significant at $P < 0.05$.

e) Evaluation of compound 1 for inhibition of binding of BMAL1 / CLOCK to E-box DNA.

i) Structure-activity relationship study

Inhibition activities of **1-18** for inhibition of binding of BMAL1 / CLOCK to E-box DNA were evaluated by the fluorescence polarization assay as described above. A droplet of compound solution (5 mM by DMSO, 1.0 μL) and another droplet of protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, 86.7 μL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 μL), the mixture (total volume 100 μL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes. The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm).

ii) Concentration-dependent inhibition activity of 1and 4

A droplet of compound solution (5 mM by DMSO, 1.0 μL) and another droplet of protein solution (BMAL1 = 37.7 μM, 1.3 μL, CLOCK = 49.1 μM, 1.0 μL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, 86.7 μL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 μL), the mixture (total volume 100 μL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes. The 96 well plate was inserted on Molecular device, and the fluorescence polarization of each sample was measured (ex = 488 nm, Em = 526 nm). In this experimental, concentrate condition of each compound was controlled for 50 μM, 10 μM, 5 μM, 1 μM, 500 nM, 100 nM, 50 nM, 5 nM.

iii) Electrophoretic mobility shift assay for Sp1ZF-GC-box DNA interaction

The complementary oligonucleotides (Invitrogen), upper (5′- Fluorescein- CGGACTTGGGGC GGGGCCTTGCTCC -3′) and lower (5′- GGAGCAAGGCCCCGCCCCAAGTCCG -3′), were annealed and used for the probe GC-box DNA. The probe DNA (5 nM), threefold serially diluted concentrations of purified Sp1ZF proteins (0-11 nM), and compound **1** (0, 1, 5, 10 μM) were mixed in the reaction buffer (20 mM Tris-HCl (pH 7.5), 70 mM NaCl, 1 mM DTT, 0.05% NP40, 10 μM ZnCl2, 5% glycerol). The mixture was incubated at 25 °C for 30 min, and free DNA and bound DNA were separated by electrophoresis on 15% non-denaturing polyacrylamide gels in TBE buffer. After electrophoresis, fluorescently labeled DNAs were detected by using a Typhoon FLA 9000 (GE Healthcare).

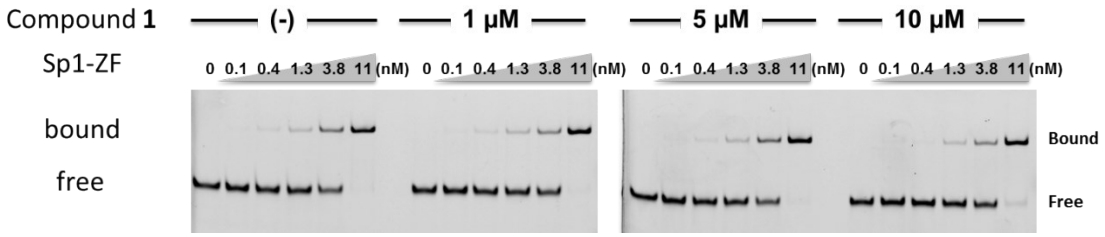


Figure S4. Electrophoretic mobility-shift (EMSA) assay of 5'-FAM-GC-box DNA (5'-FAM-CG GACTTGGGGCGGGGCCTTGCTCC-3') by the Sp1ZF-His6 (12.2 kDa; 0-11 nM) in the presence of 1 (0, 1, 5, 10 μM). The probe DNA was mixed with 1 prior to addition of the ZF protein, and the resulting mixture was incubated for 30 min at 25 °C prior to gel separation.

iv) HPLC analysis of compound 1 and 16 in aqueous solution

Compound solution droplet (5 mM by DMSO, 1 μL) was placed on 1.5 mL microtube, and buffer solution (10 mM Tris, 150 mM NaCl, pH 8.0) added and mixed by vigor of the additive agent. The solution was filtrated by using a Millex-GN (0.2 μm) filter and analyzed the physical properties of each sample by HPLC.

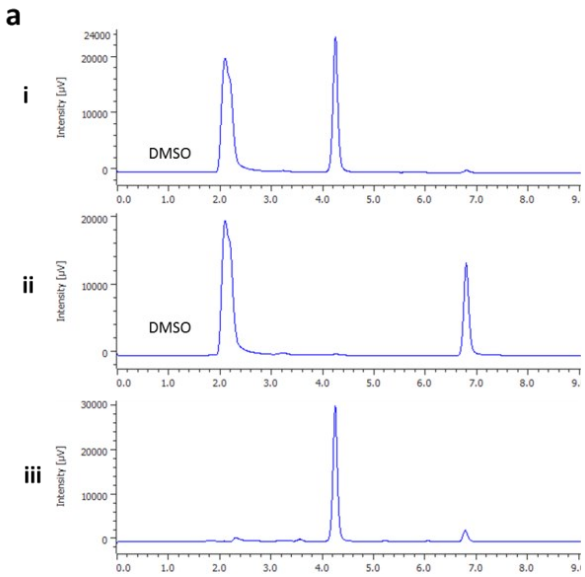


Figure S5. (a) Changes in **1** and **16** in Tris HCl buffer (10 mM Tris HCl, 150 mM NaCl, pH 8.0) were analyzed by HPLC. i) Results for a DMSO solution of **1**. ii) Results for a DMSO solution of **16**. iii) Results for **16** immediately after dissolving in a buffer solution.

v) Comparison of crystal structures of PAS B domains of HIF 2a and BMAL1

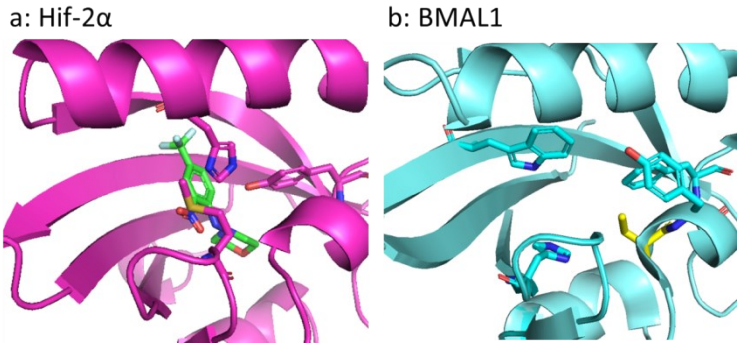


Figure S6. Crystal structures of PAS B domains. a) The cavity of PAS B domain of HIF 2α (pink ribbon) where THS044 (an inhibitor of HIF 2α, shown in stick) binds to. The hydrophobic residues were highlighted in pink rod (pdb #3f1o, [3]). b) The corresponding hydrophobic cavity of PAS B domain of BMAL1 (blue ribbon, pdb #4f3l, [4]). Typical hydrophobic residues and Cys373 were highlighted in blue and yellow rod, respectively.

vi) MALDI-TOF analysis

Proteins (BMAL1: 37.7 μM, 49.5 μL, CLOCK: 48.1 μM, 49.5 μL in 10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, 86.7 μL) were mixed with **1** (5 μM, 0.5 μM), respectively. The mixtures were incubated for 20 min at 25. Each sample was loaded into a micro dialyzer (Scienova, MWCO = 12 - 14 kDa) and placed in milliQ water (100 μL) at 4 °C for 2h. MiliQ water (~100 μL) was changed every 2 hours until the concentration of NaCl reached at 37.7 and 48.1 μM, respectively. Approximately 2 μl of each protein sample was mixed with 8 μl of saturated sinapinic acid matrix (acetonitrile 30% v/v, trifluoroacetic acid 0.1%) at 4 °C. For calibration, BSA sample was prepared by adding BSA (150 μM, 1 μL) to the matrix solution (29 μL). Each sample was placed onto a stainless-steel plate, and left at room temperature until the solution became crystal. After that, the plate was inserted into the machine and detected MW of protein respectively for MALDI-TOF.

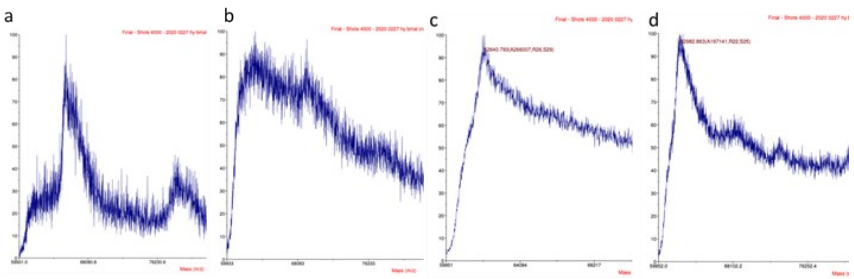


Figure S7. The results of MALDI-TOF measurement of the BMAL1 or CLOCK. (a) BMAL1 in the

absence of compound. The observed peaks are considered to correspond to BMAL1 (Calcd. Mw = 68239). (b) BMAL1 in the presence of compound (Mw = 160). (c) CLOCK in the absence of compound. The peak is considered to correspond to CLOCK (Calcd. Mw = 62104). (d) CLOCK in the presence of compound.

vii) Alkylation of BMAL1 and CLOCK using iodoacetamide

Proteins (60 µL of 27 µM of BMAL1, 40.4 µM of CLOCK) in Tris HCl buffer (10 mM Tris HCl, 150 mM NaCl, pH 8.2) were treated with iodoacetamide (3 µL of 400 mM in Tris HCl pH 8.2, BIORAD), gently voltexed, incubated at dark for 1h at r.t. The protein samples were dialyzed with microdialyzer (Scienova) against 2 ml of Tris HCl buffer at 4 °C for 2h, and this procedure was repeated three times. The proteins were subjected to DNA binding assay in a similar manner that was described above.

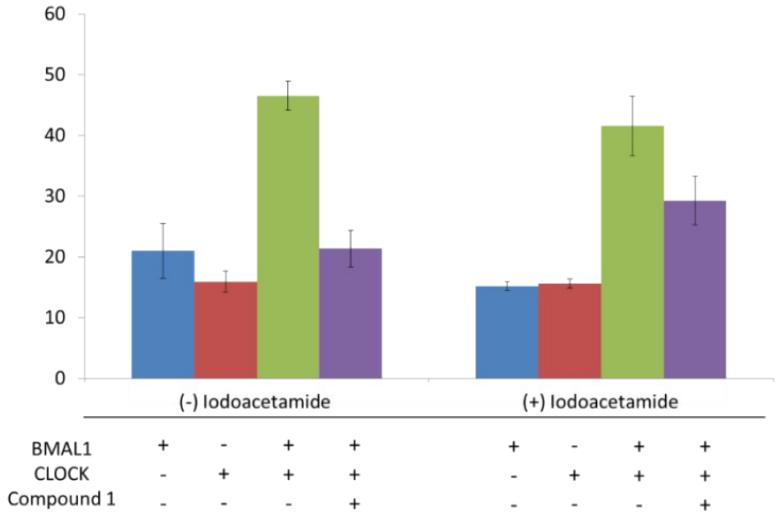


Figure S8. Left: proteins were not treated with iodoacetamide. Right: proteins were treated with iodoacetamide.

viii) SDS-PAGE analysis the BMAL1 and CLOCK after iodoacetamide treatment

A droplet of compound solution (5 mM by DMSO, 1.0 µL) and another droplet of protein solution (BMAL1 = 27 µM, 9.25 µL, CLOCK = 40.4 µM, 6.19 µL) were placed in a well of 96 well-plate respectively. To the well was added buffer solution (10 mM Tris HCl, 150 mM NaCl, and PEG200 10 % v / v, pH 8.0, 86.7 µL) added all at once to mix. After adding the dsDNA solution (100 nM, 10 µL), the mixture (total volume 100 µL) was shaken at 25 °C, for 1 min (900 rpm), and the mixture was gently incubated on a rocker at 25 °C for 19 minutes. Then BMAL1 or CLOCK in the mixture was detected by SDS-PAGE and silver stain (Wako, Silver Stain II Kit Wako).

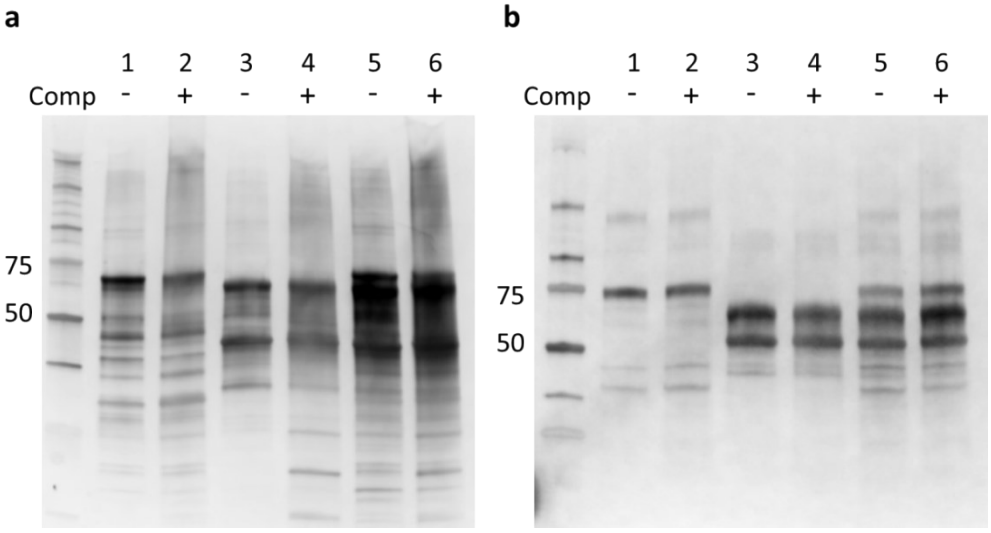
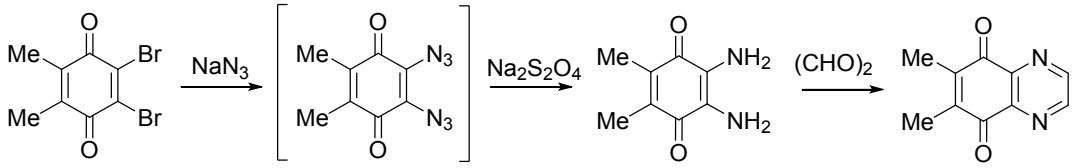


Figure S9. Left: no treatment with iodoacetamide, right: proteins were treated with iodoacetamide (20 mM). Lane 1 = BMAL1 (10 mM Tris, 150 mM NaCl, pH 8.0), lane 2 = BMAL1 treated with **1**, lane 3 = CLOCK, lane 4 = CLOCK treated with **1**, lane 5 = BMAL1 and CLOCK, lane 6 = BMAL1 and CLOCK treated with **1**. 4-12% SDS-PAGE gel (BIO-RAD, Mini-PROTEAN TGX Gels) was used in this experiment.

4) Chemistry

Among the evaluated compounds (**1**-**18**), synthetic procedures for **1**, **3**-**10**, and **12**-**18** had been reported, which were prepared according to the literatures (compounds **1**, **16**-**18**: ref. 3; compound **3**: ref. 4; compounds **4**-**5**: ref. 5; compounds **6**-**10**: ref. 6; compound 12: ref. 7; compound **13**: ref. 8; compound **14**-**15**: ref. 9). The synthetic details for the new compounds (**2** and **11**) are as follows.

Preparation of **2**



2a **2b** **2c** **2**

Starting material (**2a**) was obtained via the reported procedure (ref. 10). To a solution of **2a** (9.55 g, 32.5 mmol) in THF (100 mL) was added an aqueous solution (100 mL) of NaN_3 (26 g, 400 mmol) at 0 ºC. After stirring at 25 ºC for 3.5 h, the mixture was poured into ice-cold water with stirring to facilitate crystallization of **2b**. Dark red crystals were filtered and washed well with water. The crude product was used for the next step without further purification.

The above crystals of **2b** was dissolved in CH_2Cl_2 (150 mL) and vigorously stirred with an aqueous solution (150 mL) of $Na_2S_2O_4$ (30 g, 172 mmol) at 25 ºC for 20 h. After heating at 40 ºC for 15 min, CH_2Cl_2.was distilled out by heating the mixture at 80 ºC. Heating was continued for another 1 h. After cooling, the mixture was vigorously stirred under air at 0 ºC, aqueous NaOH solution was added portion wise until the homogeneous black suspension was obtained. After stirring for 1 h at 0 ºC, black precipitates of **2c** were filtered and washed with water. After drying and recrystallization from acetone, pure sample of **2c** (2.67 g) was obtained in 49% yield. Analytical sample was obtained by recrystallization from EtOH to give black leaflets.

To a solution of **2c** (332 mg) in 95% acetic acid (68 mL) was added 40% aqueous glyoxal (8 mL, 70 mmol), and the mixture was stirred for 3 h at 25 ºC. After 60 mL of water was added, the precipitates were filtered, washed with water and MeOH to give **2** (y. 40%) as dark orange crystals.

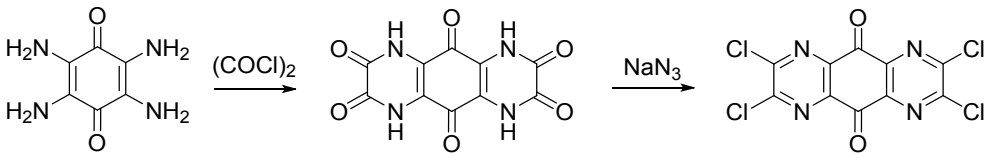
Data of **2b**: decomp. 60-65 ºC, ^{1}H NMR ($CDCl_3$, 90 MHz) 2.08 (s); IR (KBr) 2095 (N_3), 1650 (C=O) cm^{-1}.

Data of **2c**: mp 198-200 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz) 1,98 (6H, s), 3.73 (4H, br. s.); MS (EI) 166 (M^+, 100%); IR (KBr) 3440, 3343 (NH_2), 1662 (C=O) cm^{-1}.

Data of **2**: mp 225 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz) 8.67 (2H, s), 2.43(6H, s); IR (KBr) 1591

(C=O) cm^{-1}

Preparation of **11**



11a **11b** **11**

Starting material (**11a**) was obtained via the reported procedure (ref. 11). To a suspension of finely powdered **11a** (5.0 g, 30 mmol) in nitromethane (100 mL) was added oxalyl chloride (30 mL, 350 mmol). The mixture was heated at reflux for 30 h. After cooling yellow precipitates of **11b** was filtered and washed with ether. The crude product of **11b** was used without further purification. To a suspension of above precipitates of **11b** in dry DMF (100 mL), thionyl chloride (15 mL, 210 mml) was added at 0 ºC. After stirring for 1.5 h at 0 ºC. and 18 h at 25 ºC, precipitates are filtered and washed with benzene to give pure **11** (3.56 g). From the mother liquor was obtained recovered **11b**, which was similarly treated with thionyl chloride. The total yield is 49% (4.01 g).

Data of **11**: mp > 400 ºC; MS (EI) 354 (M^++4, 13%), 352 (M^++2, 24%), 350 (M^+, 100%), 248 (M^+-2, 37%); IR (KBr) 1720, 2710 (C=O) cm^{-1}.

5) References

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