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A small-molecule HDAC/PDE modulator activates human adipocyte UCP1 and resolves inflammatory signaling

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Abstract

Background

Obesity-associated inflammation in white adipose tissue (WAT) drives insulin resistance and type 2 diabetes. While UCP1-mediated thermogenesis is a therapeutic target, most browning agents lack anti-inflammatory activity. We aimed to identify small molecules that induce browning and suppress inflammation in human adipocytes.

Methods

Human cohorts and cell line models were analyzed for inflammatory markers. We generated an in-house multicomponent reaction (MCR)-based chemical library and developed a proprietary high-throughput screening platform to identify UCP1 activators in human adipocytes. Lead compound **CDC1011** was evaluated for effects on thermogenesis, mitochondrial respiration, glucose uptake, glycolysis, lipolysis, NF-κB signaling, cytokine secretion, and monocyte chemotaxis. Mechanistic studies assessed cyclic-nucleotide signaling, phosphodiesterase (PDE) inhibition, histone deacetylase (HDAC) modulation, and docking-based predictions.

Results

CDC1011 induced UCP1 expression and mitochondrial respiration, enhanced glucose uptake and lipolysis, and suppressed NF-κB activation, cytokine secretion, and monocyte recruitment. Mechanistically, CDC1011 elevated cAMP/PKA and cGMP signaling via PDE inhibition and attenuated HDAC activity, reprogramming adipocytes toward a thermogenic, anti-inflammatory phenotype.

Conclusions

CDC1011 is a first-in-class small molecule with dual thermogenic and anti-inflammatory actions in human adipocytes, offering a promising pharmacological strategy for obesity-related metabolic disorders.

Keywords

UCP1; Adipocyte browning; Inflammation; Obesity; HDAC; PDE; Thermogenesis

1. Background

Glucagon-like peptide-1 receptor (GLP-1R) agonists have emerged as a new generation of anti-obesity drugs, achieving clinically significant weight loss of approximately 15–20% [1-3]. While these agents effectively promote weight reduction and improve glycemic control by enhancing insulin secretion, suppressing glucagon levels, and delaying gastric emptying [4-6] they do not directly address the chronic inflammation and metabolic dysfunction that arise from adipose tissue remodeling in obesity. Moreover, their widespread adoption is hampered by gastrointestinal side effects such as nausea, vomiting, and diarrhea, which drive high discontinuation rates [7]. Additional concerns include sarcopenia in older adults, elevated pancreatitis risk [8,9], and loss of metabolic benefits upon cessation, with rapid weight regain and unfavorable fat redistribution. The prohibitive cost and limited

accessibility of these injectable therapies further highlight the urgent need for oral, mechanism-based alternatives that deliver sustained metabolic improvement without compromising tolerability.

Chronic low-grade inflammation in WAT is a central pathological feature of obesity, and a key driver of insulin resistance, type 2 diabetes, and cardiovascular disease [10]. This inflammatory milieu, characterized by macrophage infiltration and proinflammatory cytokine production, perpetuates a vicious cycle of adipocyte stress and systemic metabolic deterioration. In contrast, brown adipose tissue (BAT) and inducible beige adipocytes within WAT counteract energy surplus through non-shivering thermogenesis, a process governed by uncoupling protein 1 (UCP1) in the inner mitochondrial membrane. Long dismissed as vestigial in adult humans, BAT is now established as a dynamic regulator of whole-body energy expenditure, with higher activity and abundance consistently observed in lean individuals [11-15]. Activation of the thermogenic program in adipocytes not only elevates caloric dissipation but also exerts paracrine anti-inflammatory effects, making UCP1 a dual-purpose therapeutic target for obesity and its inflammatory comorbidities.

UCP1-mediated thermogenesis is tightly repressed under basal conditions by purine nucleotide binding but is robustly induced by β-adrenergic signaling or fatty acid overflow [16,17]. Pharmacological upregulation of UCP1 expression or activity thus represents a powerful strategy to reprogram energy-storing WAT into an energy-dissipating, metabolically protective phenotype. Despite intense investigation, no FDA-approved small molecule directly induces UCP1-dependent thermogenesis in humans. Prior discovery efforts have relied heavily on rodent models or immortalized preadipocyte lines, which poorly mirror human adipocyte biology, inflammatory crosstalk, or translational pharmacokinetics. These limitations have stalled clinical progress and underscore the need for human-relevant screening platforms that integrate thermogenic and immunomodulatory readouts.

Here, we bridge this translational gap by establishing a high-throughput, human adipocyte-based screening platform using a chemically diverse multicomponent reaction (MCR) library. By coupling UCP1 induction with suppression of obesity-associated inflammatory

signaling in the same cellular context, we aim to identify next-generation small molecules that simultaneously enhance energy expenditure and resolve adipose inflammation. This dual-action strategy offers a transformative framework for durable obesity management, potentially overcoming the limitations of current incretin-based therapies in metabolic disease.

2. Methods

Study Design and Participants

We enrolled 300 adults classified as normal weight (BMI <25), overweight (BMI 25–29.9), or morbid obese (BMI ≥40). Plasma samples were collected for cytokine profiling. All participants provided written informed consent. The study was approved by the Hamad Medical Corporation Research Ethics Committee and the Institutional Review Board of Weill Cornell Medicine-Qatar.

Cell Culture and Adipocyte Differentiation

Human SW872 white preadipocytes (ATCC® HTB-92™, RRID: CVCL_1730) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM/F12 medium (Thermo Fisher Scientific, 11330032), supplemented with 8% fetal bovine serum (FBS) (Thermo Fisher Scientific, 16000044), 1% penicillin–streptomycin (Thermo Fisher Scientific, 15140122), and 15 mM HEPES (Thermo Fisher Scientific, 15630080). PAZ6 preadipocytes (RRID: CVCL_GS29), generously provided by Dr. A.D. Strosberg [18], were cultured under the same conditions as SW872 cells. All cultures were maintained at 37°C in a humidified incubator with 5% CO_2. Once cells reached 70–80% confluency, they were trypsinized and sub-cultured.

For adipocyte differentiation, 10 μM oleic acid (Thermo Fisher Scientific, 031997.06) was added to SW872 cells at full confluency to induce differentiation over a 7-day period. For PAZ6 preadipocytes, differentiation was initiated at full confluency by culturing in

DMEM/F12 medium supplemented with 5% FBS, 15 mM HEPES, 33 μM biotin (Sigma-Aldrich, B4639), 17 μM pantothenate (Sigma-Aldrich, P5155), 1 nM triiodothyronine (T3) (Sigma-Aldrich, 642511), 100 nM dexamethasone (Sigma-Aldrich, D4902), 1 μM rosiglitazone (Sigma-Aldrich, R2408), and 1% penicillin-streptomycin for 14 days. In addition, for the first 4 days, 0.25 mM isobutylmethylxanthine (IBMX) (Sigma-Aldrich, I5879) was included in the differentiation media. Culture and differentiation media were refreshed every two days.

THP-1 cells, an immortalized human monocytic cell line [19], derived from an acute monocytic leukemia patient (ATCC, TIB-202™, RRID:CVCL_0006), were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in RPMI 1640 medium (Thermo Fisher Scientific, 11875093) supplemented with 10% FBS, 0.05 mM 2-mercaptoethanol (Gibco, 21985023) and 1% penicillin–streptomycin. Macrophage differentiation was induced by adding 10 nM phorbol myristate acetate (PMA) (Sigma-Aldrich, P1585) for 3 days to mimic primary human macrophages [20]. All cell lines were contamination free.

Multiplex Bead Immunoassay

Quantitative analysis of cytokines in human plasma and cell culture supernatants was performed using Cytokine Human 25-Plex kit (Invitrogen, LHC0009M) on a Luminex 200™ dual-laser detection system (Luminex Corp., CN-AY100) employing xMAP technology. This comprehensive 25-plex human cytokine panel quantifies the following proteins: GM-CSF, IFN-α, IFN-γ, IL-1ra, IL-1β, IL-2, sIL-2r, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, Eotaxin, RANTES, and TNF-α. The assay was conducted according to the manufacturer's instructions, including the recommended reconstitution of standards and proper sample dilution factors. The concentrations of unknown samples (in triplicates) were determined using a five-parameter logistic regression standard curve (fluorescence units vs. concentration in pg/mL). Cytokine concentrations in pooled plasma samples and adipocyte cell culture supernatants were plotted for analysis.

RNA Isolation and Quantitative RT-PCR

Total RNA from SW872 and PAZ6 pre-adipocytes was extracted using the Qiagen RNeasy Lipid Tissue Mini Kit (Qiagen, 74804), following the manufacturer's instructions. RNA concentration was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, ND-2000). A total of 500 ng of RNA was reverse transcribed into complementary DNA (cDNA) using GoScript Reverse Transcriptase (Promega, A5003). Quantitative PCR (qPCR) was performed in triplicate on an Applied Biosystems® QuantStudio 6 Flex Real-Time PCR System, employing GoTaq qPCR Master Mix (Promega, A6001) for SYBR Green-based detection. Gene expression was quantified using the 2-ΔCT method, with HPRT serving as the reference gene. Primer sequences are listed in Supplementary Table 2).

RNA Sequencing Reanalysis

Raw read counts and gene annotations were obtained from the NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/download/?acc=GSE63190). Data from SW872 and PAZ6 cells were extracted and processed using edgeR (version 4.0.16). The data were normalized for library size, and low-expression genes were filtered using the filterByExpr function with default parameters. Common dispersion was estimated, and differential expression analysis was performed using the exactTest function.

Migration Assay

Cell migration was assessed using a fluorometric 5-µm 24-well transwell kit (Millipore, ECM507) according to the manufacturer's instructions. Briefly, PAZ6 and SW872 cells were cultured and differentiated as described above and with treatment or vehicle. Conditioned media from the cells was collected 24 hours post incubation in serum free media and filtered at the respective time points (preadipocytes and differentiated cells). Cells were counted in each condition for normalization purposes. The conditioned media was placed in the lower chamber of the transwells, while 1×10^5 THP-1 cells were added to the upper

chamber. After 6 hours, cell migration was quantified according to the manufacturer's protocol in triplicates.

Fluorescence-Activated Cell Sorting (FACS)

Human blood-derived monocytes were differentiated into macrophages using 100 ng/mL M-CSF (PeproTech, 300-25). These macrophages were then cultured in conditioned media, treated with Brefeldin A (BD Biosciences, 555029), permeabilized, fixed, and stained with antibodies against the macrophage marker CD68 (BD Biosciences, 556595) and the macrophage inflammation marker CD36 (BD Biosciences, 550956). Expression levels were assessed using a BD Fortessa Cytometer (BD Biosciences, 650621).

Small-molecules Library

The chemical library used for screening consisted of 2186 molecules. These compounds were synthesized using various multicomponent reaction (MCR) methodologies, enabling the creation of a diverse chemical library suitable for high-throughput screening. The compounds were stored as 100 mM stock solutions in DMSO. The synthetic approaches involved multiple MCR types, including Ugi, Passerini, Groebke-Blackburn-Bienaymé, tetrazoles, covalent warheads, and macrocyclization reactions [21-26].

Chemoinformatic Analysis

All analyses were performed in Python (v3.13) with the following key libraries: RDKit (v2024.03.4) for molecular descriptor generation, scikit-learn (v1.5.0) for data standardization and Principal Component Analysis (PCA), Pandas (v2.2.2), NumPy (v1.26.4) for array manipulations and numerical integration, SciPy (v1.15.1) for hierarchical clustering and statistical tests, and Matplotlib (v3.8.4) together with Seaborn (v0.13.2) for data visualization. All molecular structures depicted in this study were

rendered using PyMOL (Schrödinger, LLC). Details of the chemoinformatic analysis are provided in the Supplementary Material (pages 3-10).

Library Screening Platform

Two distinct lengths of the human UCP1 promoter region (4 kb and 6 kb upstream of the transcription start site) were cloned upstream of a luciferase reporter gene in the PGL4.15 vector (Promega, E6701). A human BAC clone (CTD-2211H19) was obtained from Invitrogen. BAC DNA was purified using the PureLink™ HiPure Plasmid Maxiprep Kit (Invitrogen, K210005).

The 4 kb and 6 kb UCP1 promoter regions were amplified using KAPA HiFi HotStart DNA Polymerase (Roche, KK2502) with the following primer pairs:

- 4Kb:
  - Forward:5'-CTCGAGTGTGCAGCGATTTCTGATTG-3'
  - Reverse: 5'-AGATCTTCACTCAGAGACTGGAGATGC-3'
- 6Kb:
  - Forward:5'-CTCGAGTCCTTTTCACTCTCTGGTCCTTC-3'
  - Reverse: 5'-AGATCTACTCAGAGACTGGAGATGCAGA-3'

These promoter regions were cloned into the PGL4.15 vector using the XhoI and BglII restriction sites. The constructs were then transfected into SW872 cells and PAZ6 cells using Lipofectamine™ 2000 (Invitrogen, 11668019). Successful transfection was confirmed, and stable cell lines expressing the constructs were selected with 50 μg/mL of hygromycin (Invitrogen, 10687010). Luciferase activity was further validated in the stable lines.

Small Molecule Library Screening

For initial hit identification, a library of 2186 small molecules was screened in the SW872 and PAZ6 cell lines. The screening was performed in triplicate with six technical replicates per experiment. Briefly, SW872 and PAZ6 pre-adipocytes were seeded in 96-well plates (Corning, 3610) at a density of 10,000 cells per well and allowed to adhere for 24 hours. Each compound (10 μM) was added for 24 hours, followed by a luciferase assay using the Luciferase Assay System (Promega, E1501) according to the manufacturer's protocol. Luciferase activity was measured using a CLARIOstar plate reader (BMG Labtech, 0412-101). The luciferase signal from each compound treatment was normalized to the basal response in the presence of DMSO.

Cell Viability Assay

Cell viability was assessed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, G7571) following the manufacturer's instructions. Briefly, cells were seeded in 96-well opaque-walled plates at a density of 10,000 cells per well. After 24 hours, cells were treated with the compounds (10 μM) for 24 hours. The plate was equilibrated at room temperature for 30 minutes, and an equal volume of CellTiter-Glo reagent was added to the medium. After mixing for 2 minutes, the plate was incubated for an additional 10 minutes. The luminescent signal was then measured using a CLARIOstar plate reader.

Dose-Response Assay

To generate dose-response curves, PAZ6 cells were seeded in 96-well plates at a density of 10,000 cells per well and allowed to adhere overnight. Serial dilutions of test compounds were prepared starting at a concentration of 10 μM, followed by 2-fold serial dilutions to achieve six concentrations. Each condition was tested in five technical replicates. Equal volumes of compound solutions were added to the wells to maintain consistency. After 24 hours of treatment, the medium was aspirated, and the cells were washed twice with 100 μL of PBS. Cells were then lysed with 20 μL of Cell Culture Passive Lysis Reagent (Promega, E1941). Luciferase activity was quantified using the Luciferase Assay System (Promega,

E1501) according to the manufacturer's instructions. Data were analyzed to determine EC50 values by fitting the dose-response data to a sigmoidal dose-response curve using GraphPad Prism software.

Western Blotting

Western blot analysis was performed on undifferentiated and differentiated SW872 cells as described above and with treatment or vehicle. Cells were lysed using Pierce™ RIPA Lysis Buffer (Thermo Scientific, 89901) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, 78442). Protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, 23227). 25ugs of Proteins were mixed with 4X Laemmli sample buffer (Bio-Rad, 1610747) and heated at 95°C for 5 minutes. Samples were separated by SDS-PAGE, transferred to PVDF membranes (Millipore, IPVH00010), and blocked with 5% Bovine Serum Albumin (BSA) Blocking Buffer (Sigma-Aldrich, A3059). Membranes were incubated with primary antibodies against UCP1 (ABclonal, A5857), Cidea (Prosci, 2089), β3AR (ABclonal, A8607), Eva1(Proteintech, 11787-1-AP), Vinculin (ABclonal, A2752), OxPhos Human Antibody Cocktail (Invitrogen, 45-8199), PDE5A (Santa Cruz Biotechnology, sc398747), Phospho-NF-κB p65 (Ser536) (Cell signaling, 3033S), GAPDH (Cell signaling, 2118S), Actin (Cell signaling, 3700S), or α-tubulin (Proteintech, 66031-1). After washing, membranes were incubated with secondary antibodies (Cell signaling, 7074S, 7076S), and chemiluminescence was developed using Immobilon Crescendo Western HRP substrate (Millipore, WBLUR0500). Signal was captured using a ChemiDoc Imaging System.

Cellular Bioenergetics Assay

Cellular respiration was measured using a Seahorse XF96 Analyzer (Seahorse Bioscience, Agilent Technologies) as previously published [27]. SW872 adipocytes were seeded at 10,000 cells/well in XF96 microplates (Agilent Technologies, 101085-004) and differentiated in the presence of vehicle (DMSO) or compounds throughout the

differentiation period. On day 7, oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using the XF Mito Cell Stress Test (Agilent Technologies, 103015-100), XF Long Chain Fatty Acid Oxidation Stress Test (Agilent Technologies, 103693-100) and Glycolysis Stress Test Kit (Agilent Technologies, 103020-100), following the manufacturer's protocols. Prior to the assay, cells were incubated in XF assay medium containing 2 mM glutamine (Agilent Technologies, 103579-100), 10 mM glucose (Agilent Technologies, 103577-100), and 1 mM pyruvate (Agilent Technologies, 103578-100) at 37°C in a CO_2-free atmosphere. Drugs were injected into the XF sensor cartridge as per the specific protocols for each test. The following inhibitors were used: Oligomycin; carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP); Rotenone+Antimycin A. For the Long Chain Fatty Acid Oxidation Stress Test, cells were incubated in substrate-limited growth media consisting of base FAO medium media supplemented with 0.5mM glucose, 1mM glutamine, 1% FBS and 0.5mM L-Carnitine 24 hours before the assay. Prior to the assay, cells were switched to fatty acid oxidation medium containing a defined ratio of XF Palmitate-BSA (6:1) and XF BSA as the only extracellular fuel source for the cells. Media or Etomoxir was injected in port A prior to Oligomycin, FCCP and Rotenone + Antimycin. For the Glycolysis Stress Test Kit, cells were incubated with XF assay medium without glucose or pyruvate. Glucose, oligomycin and 2-deoxy-glucose (2-DG) are sequentially injected. To assess mitochondrial function, OCR and ECAR data were normalized to representative cell counts, determined by nuclei staining with Hoechst 33342 (Thermo Scientific, H3570) imaged on an ImageXpress and analyzed using MetaXpress software (Molecular Devices). The data were analyzed and plotted using XFe-96 software (wave) and the Seahorse XF Cell Mito Stress Test Report Generator following the manufacturer's instructions (Agilent, Inc).

Cytokine Assay

SW872 cells were seeded at 350,000 cells/well in 6-well plates and treated with vehicle (DMSO) or compounds in the presence or absence of oleic acid (OA) at 10 μM. After 7 days, cell culture supernatants were collected and centrifuged to remove particulates. Cytokine

profiles were analyzed using the Proteome Profiler Array, Human XL Cytokine Array Kit (R&D Systems, ARY022B) following the manufacturer's protocol. Briefly, cell culture supernatants were diluted and incubated with the Cytokine Array overnight. After washing, the membranes were incubated with a cocktail of biotinylated detection antibodies. Chemiluminescence detection was performed using streptavidin-HRP and chemiluminescent reagents, with signals captured using a ChemiDoc Imaging System. Spot quantification was performed using ImageJ software, and cytokine levels were normalized to cell count.

RNA Sequencing

https://digitalinsights.qiagen.com/IPASW872 cells were seeded at 350,000 cells/well in 6-well plates and treated with vehicle (DMSO) or compounds for the entire differentiation period. RNA was extracted at day 2 and day 7 using the PureLink RNA Mini Kit (Thermo Fisher Scientific, 12183018A) according to the manufacturer's protocol. RNA quality and quantity were assessed using the Quant-iT™ RiboGreen RNA Assay Kit (Thermo Fisher Scientific, R11490). Sequencing libraries were prepared by Genomics Core at WCMQ using the Lexogen CORALL Total RNA-Seq V2 Kit (Lexogen, 016.24) according to the manufacturer instructions. Samples were then barcoded, pooled and sequenced (PE 150bp) in a single run in the same lane on the NextSeq 550 HO platform (Illumina). Alignments and gene counts were done with STAR [28] (version 2.7.10a) to reference GRCh38 using Gencode (v41) gene annotations. Read counts per gene were obtained with STAR with a read counted if it overlaps with one and only one gene. Differential expression was performed with edgeR (version 4.0.16) [29] after filtering genes with low read counts (at least two reads in 10% of samples with a minimum of five reads across samples), TMM normalization and estimating dispersion (default parameters). The Benjamini-Hochberg procedure was used to control false discovery. The edgeR function cpmsByGroup was used to transform read counts to counts per million (cpm) for clustering and pathway analysis. Quality control was assessed using PCA. Pathway analysis and upstream regulator analysis were performed using Ingenuity Pathway Analysis (IPA) (QIAGEN Inc.,) [30] and Gene Set

Enrichment Analysis (GSEA) 4.3.3 software [31,32]. The differential gene expression data were filtered for p value < 0.05 and 0.7 fold change (FC) and were further analyzed in the context of known biological response and regulatory networks using Ingenuity Pathways Analysis (IPA, Ingenuity® Systems, www.ingenuity.com). Two metrics were used to identify upstream regulators of differentially expressed genes: z-score and p-value. The z-score algorithm was used to predict the direction of change for a given function (increase or decrease). A positive z-score (≥ 2) indicates significantly increased functional activity whereas a negative z-score (≤ 2) indicates a significantly decreased functional activity in the CDC1011-treated cells. For GSEA analysis an FDR cutoff of 25% was used based on the recommendation for phenotype permutation.

Lipolysis Assay

SW872 cells were seeded in 96-well plates at a density of 10,000 cells/well and treated with differentiation media containing vehicle or compounds at 10 μM. After 7 days, cells were incubated in serum-free media for 24 hours, and lipolysis was induced by incubation with Krebs-Ringer Bicarbonate (KRB) buffer containing 5 mM glucose, 4% BSA, and Forskolin (5 μM, F6886) or Isoproterenol (10 μM, Sigma-Aldrich, I6504) for 2 hours. Glycerol levels were measured using the Glycerol-Glo™ Assay (Promega, J3150) according to the manufacturer's protocol. Glycerol concentrations were normalized to cell viability, assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7571).

Glucose Uptake Assay

Glucose uptake was measured using the Glucose Uptake-Glo™ Assay (Promega, J1341). SW872 cells were seeded at 10,000 cells/well in 96-well plates and subjected to differentiation or propagation media and treated with vehicle or compounds at 10 μM for 7 days. Prior to the assay, cells were incubated in serum-free medium for 24 hours, followed by incubation in glucose-free media for 1 hour. After treatment, glucose uptake was

quantified and normalized to cell viability using the CellTiter-Glo® Luminescent Cell Viability Assay.

cAMP and cGMP ELISA

SW872 cells were seeded at 350,000 cells/well in 6-well plates and subjected to differentiation or propagation media with vehicle or CDC1011 for 7 days. At day 7, cells were incubated in serum-free media for 24 hours, followed by 4-hour treatment with IBMX (0.25 mM, Sigma-Aldrich I7018) and Forskolin (10 μM, Sigma-Aldrich, F6886). cAMP and cGMP levels were measured using the Cyclic AMP Select ELISA Kit (Cayman, 501040) and the Cyclic GMP ELISA Kit (Cayman, 581021) according to the manufacturer's protocols. The concentrations of cAMP and cGMP were normalized to the protein content in each condition.

PKA Activity Assay

Protein Kinase A (PKA) activity was assessed using the PKA Colorimetric Activity Kit (Invitrogen, EIAPKA) following the manufacturer's protocol. Briefly, cell lysates were incubated with ATP, and after washing, PKA substrates were detected using the donkey anti-rabbit IgG HRP Conjugate and Phospho-PKA Substrate Antibody. The reaction was developed and quantified at 450 nm. Values were normalized to the protein content.

Molecular Docking

Ligand–receptor docking was performed using the ROSIE web server implementation of RosettaLigand [33-36]. The crystal structure of HDAC isoforms were uploaded in PDB format; water molecules and unrecognized small entities were automatically removed. Protonation state was adjusted to pH 7.4 with Open Babel [37]. Ligands were provided as SDF files with explicit hydrogens and 3D coordinates. Conformational diversity was ensured by generating up to 200 ligand conformers with the BioChemical Library (BCL)

toolkit. Docking followed a two-stage Monte Carlo-based protocol. During the low-resolution phase, 500 sampling steps were executed within a 5 Å radius from the ligand's initial position, which was further randomized within a 3 Å sphere. The low-resolution grid size was set to 15 Å, with translational and rotational steps of 0.1 Å and 5°, respectively. This was followed by six cycles of high-resolution docking, during which side-chain repacking occurred every third cycle. A total of 200 docking models (decoys) were generated for each ligand. Final poses were ranked based on the Rosetta interface and total energy scores, and the top-scoring conformations were retained for further analysis. All parameters were used as recommended in the ROSIE documentation and are consistent with previously validated RosettaLigand protocols. Images were generated in PyMOL (Schrödinger, LLC), and a detailed analysis of non-covalent protein–ligand contacts (e.g., hydrogen bonds, hydrophobic interactions, π–π stacking) and cooperativity binding network analysis were performed with Scorpion (DesertSci SAAS platform) [38].

Histone Deacetylase Activity

Histone Deacetylase (HDAC) activity was assessed using HDAC Activity Fluorometric Assay Kit (ab156064) following the manufacturer's protocol. Briefly, HDAC assay buffer and substrate are added to wells of a 96 well plate, then vehicle (DMSO), CDC1011 (5μM), and Trichostatin A (1 μM, a known HDAC inhibitor) were added to different wells and mixed thoroughly. This is followed by adding crude HDAC enzymes and incubated for 20 minutes. Signal in each well was developed, and fluorescence intensity was measured at Ex/Em = 355/460 nm.

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software (GraphPad). Statistical significance was determined using the Student's t-test with a significance threshold of $P \leq 0.05$. For statistical comparisons of the molecular descriptor distributions, a Mann-Whitney U test

was applied whenever the Shapiro-Wilk test indicated a non-normal distribution. For the fingerprint CDF analysis descriptive statistics (mean, median, standard deviation) were calculated for each set of similarity values. Welch's t-test (with equal_var=False) was then applied pairwise across the three datasets to evaluate whether their distributions of similarity scores differed significantly. The degrees of freedom were approximated for reporting purposes, and p-values < 0.05 were considered statistically significant. For ECDF distributions a two-sample Kolmogorov–Smirnov test was performed pairwise to assess statistical differences among the three datasets.

3. Results

Proinflammatory cytokine enrichment in obesity and white adipocytes

To delineate the proinflammatory milieu in obesity, we profiled plasma cytokines in 100 morbidly obese individuals (BMI ≥ 40), 100 overweight individuals (25 ≤ BMI < 30), and 100 normal-weight controls (BMI < 25) using multiplex bead immunoassay. Of 25 cytokines assayed, soluble interleukin-2 receptor (sIL-2R) was elevated in both overweight and obese groups relative to lean controls, whereas interferon-alpha (IFN-α), IL-6, IL-12, monocyte chemoattractant protein 1 (MCP1), and interferon-inducible protein 10 (IP-10) increased specifically in obesity (Figure 1a). Levels of IFN-α, IL-6, IL-12, and sIL-2R displayed a progressive gradient from normal weight through overweight to obesity.

Further exploring the cellular basis of this inflammatory response, we assessed the secretion of pro-inflammatory cytokines during *in vitro* adipogenesis of human white adipocytes (SW872) and brown adipocytes (PAZ6). Consistent with the findings in human plasma, mature white adipocytes secreted higher levels of IFN-α, IL-6, IL-12, MIP-1α/β, and IL-7, while the maturation of brown adipocytes led to a significant reduction in the secretion of these cytokines (Figure. 1b).

Figure 1. Proinflammatory cytokine enrichment in obesity and white adipocytes. **a** Multiplexed Elisa of pro-inflammatory markers in plasma of obese subjects and overweight subjects compared to lean controls. **b** Multiplexed Elisa of pro-inflammatory markers done on supernatant from white adipocytes SW872 cells at day 0 and day 7 of differentiation and brown adipocytes PAZ6 cells at Day 0 and Day 14 of differentiation. **c** IPA analysis of differentially expressed inflammatory-related genes in undifferentiated versus differentiation SW872 and PAZ6 cells. The genes are placed relative to their cellular localization. The color of each gene reflects it differential relative expression in differentiated versus undifferentiated PAZ6 cells where green is downregulated and red is upregulated. The bar plot adjacent to each gene display its differential expression in both PAZ6 (left) and SW872 (right) cell lines respectively in differentiated versus undifferentiated state. Direct interactions are depicted by solid gray lines and indirect interactions by dashed gray lines. **d** THP-1 migration towards conditioned media derived from pre-differentiated and differentiated SW872 and PAZ6 adipocytes. **e** FACS analysis of CD36 levels in blood-derived-macrophages treated with conditioned media from pre-differentiated and differentiated SW872 and PAZ6 adipocytes (N=3). SW, SW872; CTRL, control; OW, overweight; OB, obese, Pre, pre-differentiated; Diff, differentiated. All data are presented as mean ± S.D. p values were calculated by unpaired two-tailed Student's *t* test. ms, marginally significant (0.05<P<0.08); *, P<0.05; **, P<0.01; ***, P<0.001

Reanalysis of RNA-sequencing data from pre- and mature SW872 and PAZ6 adipocytes [39] revealed extensive transcriptomic shifts (Figure. 1c and Supplementary Figure 1a-c). Pathway enrichment in SW872 highlighted metabolic, mitochondrial, and cellular processes, while PAZ6 showed immune pathway downregulation during maturation (Figure 1c and Supplementary Figure 1b, -c).

qPCR validation confirmed downregulation of IL-6, IL-8, TNF-α, CXCL5, IL-1α, IL-1β, and CXCL6 in mature brown adipocytes, contrasting upregulation in white adipocytes

(Supplementary Figure 1d). Immunofluorescence corroborated reduced IL-6 and IL-8 in differentiating PAZ6 versus increased in SW872 (Supplementary Figure 1e).

These data underscore the distinct pro-inflammatory profiles of brown and white adipocytes. Specifically, white adipogenesis is associated with an elevated pro-inflammatory signature.

White adipocytes promote monocyte recruitment and M1 polarization

Adipose-resident macrophages amplify inflammation in obesity [40]. We assessed THP-1 macrophage migration toward supernatants from pre- and mature SW872/PAZ6 adipocytes. Mature white adipocyte supernatants potently induced migration, exceeding preadipocyte or brown adipocyte equivalents (Figure 1d).

Further, exposure to mature SW872 supernatants upregulated CD 36, a M1 proinflammatory marker [41], in blood-derived macrophages, unlike PAZ6 supernatants (Figure 1e). This underscores white adipocytes' secretion of chemokines that recruit and polarize macrophages to proinflammatory states, fueling obesity-linked metaflammation.

MCR library design and diversity

Given the above results and evidence from various studies regarding the high inflammatory profile associated with WAT relative to BAT, adipocyte browning has emerged as a promising therapeutic approach for obesity and its associated inflammation and metabolic dysfunction [42-45]. Moreover, multiple studies uncovered an unexpected anti-inflammatory role of the key browning marker, UCP1, in different tissues [46-49]. However, a major hurdle in this area is the identification of pharmacological agents that can effectively and safely induce adipocyte browning. To overcome this challenge, we developed a high-throughput screening platform and an unconventional MCR library of small molecules aimed at identifying novel compounds capable of inducing UCP1 expression in human adipocytes.

Chemoinformatic comparison of the MCR library against FDA-approved drugs [50,51] and cellular metabolites evaluated physicochemical properties (Supplementary Tables 4,5). The library showed higher molecular weight, lipophilicity, and lower topological polar surface

area (TPSA) than metabolites, with reduced hydrogen-bond donors/acceptors implying enhanced permeability but altered solubility [52]. Detailed analyses (e.g., PCA, shape, fingerprints, scaffolds) (Supplementary Tables 6-10)confirmed the library's diversity and novelty, partially overlapping FDA drugs but diverging from metabolites, suggesting access to novel biological targets.

High-throughput screening identifies UCP1 inducers

Having established the chemical and biophysical properties of the MCR library, we proceeded with a high-throughput luciferase-based screening platform to identify small molecules capable of activating UCP1 expression in human adipocytes. We generated stable human adipocyte cell lines expressing a luciferase reporter driven by the human UCP1 promoter. We constructed two variants of the reporter system: one with the complete ~6Kb promoter region and another with a truncated ~4Kb version (Figure 2a). Given the physiological differences between rodent and human obesity models [53,54], we chose to establish a human cell-based system for the screening process. We first confirmed the functionality of the reporter system by treating the cells with known UCP1 activators, including rosiglitazone and Genistein [55,56]. Both compounds resulted in a significant increase in luciferase activity in cells with both 4Kb and 6Kb UCP1 promoters (Figure 2b). The 6Kb promoter construct resulted in higher luciferase activity (Figure 2b), and since SW872 cells (human white adipocyte line) proliferate more rapidly than PAZ6 (brown adipocyte line), we chose to use the SW872 reporter cells with the 6Kb UCP1 promoter for the high-throughput screening.

Figure 2. A high-throughput small compound-UCP1 inducers screening platform. **a** Diagram of the establishment of screening platform showing two human UCP1 promoter regions (4 K and 6K) inserted above the luciferase reporter, respectively. **b** Validation of screening platform through luciferase activity induction in SW872 (upper panel) and PAZ6 (lower panel) treated with known UCP1 inducers, rosiglitazone (ROS) and genistein (GEN).

UNT, untransfected; CTRL, control. **c** Screening of an MCR compound library in SW872 preadipocytes with UCP1 luciferase reporter (N=3). Luciferase signal fold change was calculated relative to DMSO-treated cells after 24 hours of compound treatment. **d** Secondary screen of the candidates in SW872 preadipocytes. Luciferase signal fold change in SW872 preadipocytes after 24 hours of treatment with the hits from the primary screen relative to the control (N=3). **e** Luciferase signal fold change in PAZ6 cells treated with candidate compounds identified in the SW872 screen (N=3). **f** Cell viability (measured by CellTiter-Glo luminescence) of SW872 preadipocytes treated with the indicated compounds (at 10uM) or vehicle for 24 hours (N=3). **g** and **h** Relative mRNA expression level of UCP1(g) and ADRB3 (h) in SW872 differentiated white adipocytes upon treatment with 10 μM of candidate compounds and vehicle (N=3). The results of the replicates and independent experiment analyses were presented as mean ± S.D. Statistical analyses have been performed using Student's *t* test. *, P<0.05; **, P<0.01; ***, P<0.001

The high-throughput luciferase screening of the MCR library revealed several compounds that significantly increased luciferase activity compared to the control, indicating their potential as UCP1 inducers (Figure 2c). These initial hits were subjected to three independent validation experiments using SW872 cells (Figure 2d). Based on consistent results, nine candidates were identified that significantly enhanced UCP1 expression in both SW872 and PAZ6 cells (Figure 2e). To assess the impact of these candidates on cell viability, we tested them in both SW872 and HepG2 cells. The results showed that none of the nine candidates majorly impact cell viability in SW872 cells, with less than a 7% reduction in cell viability (Figure 2f). Similarly, the compounds did not affect HepG2 cell viability, except for one compound, CDF48, which reduced viability by 12% (Supplementary Figure 4). These findings suggest that the identified UCP1 inducers are well-tolerated by adipocytes and do not pose significant toxicity risks. Dose–response evaluations for each of

the nine candidates were performed using the luciferase reporter system in PAZ6 cells, which revealed a clear dose-response effect (Supplementary Figure 5).

We then examined the endogenous expression of UCP1 in differentiated SW872 adipocytes treated with each of the nine candidates. Quantitative PCR (qPCR) analysis showed that two compounds, CDC1011 and CDCF911, significantly upregulated the transcriptional expression of UCP1 in differentiated SW872 adipocytes (Figure 2g). In addition, we assessed the expression levels of Beta-3 adrenergic receptor (ADRB3) in mature SW872 adipocytes after treatment with the various compounds. ADRB3 is a key receptor involved in thermogenesis and is one of the primary signals for triggering the beiging process of mature white adipocytes, leading to improved metabolic function and potential weight loss [57-59]. Previous studies have demonstrated that moderate increases in ADRB3 expression are associated with body mass reduction [60-62]. Our results revealed that only CDC1011 induced a moderate increase in ADRB3 expression in mature SW872 adipocytes (Figure 2h), highlighting its potential as a browning inducer. Based on these findings, we selected CDC1011 for further analysis.

Chemoinformatic analysis of CDC1011 revealed no substructural analogues in public bioactive databases such as the Protein Data Bank (PDB) and ChEMBL, suggesting that the structure is novel. CDC1011 possess a molecular core of (1R)-1-(1H-1,2,3-Triazol-1-yl)-1-(pyridin-3-yl)-2-phenylethan-1-amine, and this core structure incorporates a tetrazole moiety, known for its resistance to amidase metabolism and potential to enhance pharmacokinetic properties [63]. Furthermore, CDC1011 also features a quinoline ring. The quinoline ring is well-known for improving bioactivity and pharmacokinetics, enhancing metabolic stability and membrane permeability, and facilitating π-π stacking interactions with molecular targets [64]. SwissADME for CDC1011 reports XLOGP3 of 2.05, molecular weight of 316.36 $g \cdot mol^{-1}$, TPSA of 82.5 $Å^2$, ESOL log S of −3.48 (0.106 mg mL^{-1}, class Soluble), four rotatable bonds, and fraction Csp^3 of 0.11. Rule-based filters show no violations under Lipinski, Ghose, Veber, Egan, and Muegge, the bioavailability score is 0.55, there are no PAINS or Brenk alerts, and the synthetic accessibility is 3.23. CYP predictions indicate inhibition of CYP2C19 while 1A2, 2C9, 2D6, and 3A4 are negative, and the predicted skin permeation log Kp is -6.77 $cm \cdot s^{-1}$. On the radar axes the profile lies within the drug-like region for lipophilicity, size, polarity, solubility, and flexibility, whereas the low

Csp^3 indicates low saturation. Overall the data are consistent with oral lead-likeness, with increasing saturation (higher Csp^3) as the primary optimization direction.

CDC1011 induces browning and metabolic reprogramming

We next sought to confirm that CDC1011 activates the thermogenic program in adipocytes by assessing the expression of proteins linked to the brown and beige adipocyte profiles. In SW872 preadipocytes, CDC1011 treatment led to increased expression of UCP1, Beta-3 adrenergic receptor (β3-AR), EVA1, and CIDEA (Figure 3a). Similarly, in mature SW872 cells, CDC1011 treatment during differentiation resulted in upregulated expression of UCP1, β3-AR, EVA1, and CIDEA (Figure 3a). These findings strongly indicate that CDC1011 has the potential to promote the differentiation of white adipocytes toward a brite (brown-in-white) adipocyte-like phenotype.

Figure 3. CDC1011 induces the browning of white adipocytes. **a** Effect of CDC1011 treatment on browning markers in preadipocytes and differentiated SW872 adipocytes. **b** Effect of CDC1011 treatment on mitochondrial electron transport chain subunit protein expression in preadipocytes, differentiated adipocytes, and isoproterenol-induced differentiated adipocytes. Presented results are representative of biological replicates (N=3). **c** cAMP content in control and CDC1011-treated preadipocyte and differentiated adipocyte. The values were measured by ELISA and normalized to the protein content. **d** Effect of CDC1011 treatment on basal and forskolin-induced PKA activity in preadipocytes and differentiating adipocytes. The values were normalized to the protein content. **e** Effect of CDC1011 treatment on basal and isoproterenol/forskolin-induced intracellular glycerol levels in differentiating adipocytes. The results of the replicates and independent experiment analyses (N=3) were presented as mean ± S.D. Statistical analyses have been performed using Student's *t* test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Given the high oxidative capacity of brown adipocytes, we investigated the effect of CDC1011 on the expression of subunits of the mitochondrial electron transport chain, which are abundant in brown adipocytes. Treatment with CDC1011 resulted in the upregulation of several subunits of the mitochondrial electron transport chain (complexes I to V) (Figure 3b and Supplementary Figure 6a), further supporting the conclusion that CDC1011 induces a thermogenic, brown adipocyte-like molecular signature in white adipocytes.

In addition to promoting browning, thermogenesis in adipocytes is often coupled with lipolysis, mediated by β3-AR stimulation and cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) activation [65,66]. We evaluated the effect of CDC1011 on lipolysis by measuring cAMP levels, PKA activity, and glycerol content in adipocytes. Basal cAMP levels were significantly increased in both preadipocytes and mature adipocytes upon CDC1011 treatment (Figure 3c). PKA activity was also enhanced in both preadipocytes and mature adipocytes treated with CDC1011, both at basal levels and after induction with Forskolin (Figure 3d). Furthermore, differentiated SW872 adipocytes treated with CDC1011 exhibited increased intracellular and extracellular glycerol content upon stimulation with isoproterenol or forskolin compared to controls (Figure 3e and Supplementary Figure 6b), indicating that CDC1011 activates lipolysis in adipocytes via β3-AR stimulation and the cAMP/PKA signaling pathway.

Collectively, these data demonstrate that CDC1011 induces both the browning and thermogenic programs in white adipocytes, activating key molecular and physiological pathways that promote energy expenditure and fat metabolism.

CDC1011 treatment enhances mitochondrial respiration and glycolytic flux

Following the observed cAMP/PKA-induced lipolysis and expression of thermogenic markers in CDC1011-treated white adipocytes, we next explored whether this thermogenic

phenotype is associated with elevated and uncoupled mitochondrial respiration. To address this, we performed a mitochondrial stress test on adipocytes, maintaining them under normal substrate conditions or switching to a glucose-free medium 24 hours prior to the assay.

The oxygen consumption rate (OCR) parameters of CDC1011-treated adipocytes were significantly higher than those of controls under both glucose and glucose-free conditions. These treated adipocytes exhibited higher basal respiration, increased spare respiratory capacity, enhanced ATP production, and higher FCCP-induced maximal respiration compared to the controls. Additionally, CDC1011 treatment led to increased proton leak and non-mitochondrial respiration (Figure 4a). The increased uncoupled respiration mediated by proton leak confirmed the functionality of UCP1 in CDC1011-treated cells, supporting the distinct metabolic capacity of these thermogenic adipocytes.

Figure 4. CDC1011 treatment enhances mitochondrial respiration and glycolytic flux. **a** Oxygen consumption rates (OCR) diagrams of Seahorse Mito stress assay results on differentiated SW872 treated with vehicle and CDC1011 in real time under basal and starvation conditions and in response to oligomycin, FCCP and antimycin A/rotenone injected at the indicated time points with the quantification of basal and maximal respiration, proton leak, spare capacity, non-mitochondrial respiration and ATP production. Assay results were normalized to cell count in a representative field. **b** Normalized OCR diagrams for FAO stress test obtained in BSA- and Palmitate- treated SW872 adipocytes, with or without Etomoxir. Measurements were taken for differentiated adipocytes treated with vehicle or CDC1011. **c** Extracellular acidification rates (ECAR) and normalized glucoPER diagram for glycolysis stress test conducted to evaluate responses of vehicle and CDC1011 treated adipocytes to glucose and the glycolytic inhibitor 2-DG. Basal and induced glycolysis are calculated and shown. **d** Basal and insulin induced glucose uptake in preadipocyte and differentiating SW872 cells upon treatment with CDC1011 compared to

vehicle. All data is presented as mean ± S.D. and representative of independent replicates (N=3). p values were calculated by unpaired two-tailed Student's *t* test. *, P<0.05; **, P<0.01; ***, P<0.001

Next, we investigated whether CDC1011 induces changes in the fatty acid oxidation (FAO) capacity of mature adipocytes by measuring OCR in the presence and absence of exogenous substrate-palmitate (the most common long-chain fatty acid), with and without blocking the transport of long-chain fatty acids (FAs) into mitochondria. Without added exogenous fatty acids, both basal and non-mitochondrial respiration as well as proton leak were significantly higher in CDC1011-treated adipocytes compared to vehicle-treated adipocytes (Figure 4b). Importantly, the addition of palmitate did not significantly alter cellular bioenergetics in either group (Figure 4b), suggesting that both treated and untreated adipocytes primarily rely on endogenous fatty acids released under basal conditions rather than exogenous palmitate. However, CDC1011-treated adipocytes exhibited higher basal respiration and proton leak compared to controls even when exogenous fatty acids were provided (Figure 4b). This increase likely reflects CDC1011-induced intracellular lipolysis, leading to higher availability of endogenous fatty acids for oxidation.

We also assessed the effect of CDC1011 on the ability of the cells to oxidize fatty acids when FA transport into the mitochondria was blocked using Etomoxir, a known inhibitor of the carnitine palmitoyltransferase 1 (CPT1) enzyme. Even when FA transport was blocked and cells were forced to utilize alternative substrates, CDC1011-treated cells still showed enhanced basal respiration, proton leak, and non-mitochondrial respiration regardless of whether exogenous palmitate was present (Figure 4b). While maximal respiration was unaffected, spare respiratory capacity was reduced in the CDC1011-treated group. These results suggest that the effects of CDC1011 are independent of fatty acid entry into mitochondria and that CDC1011 promotes the use of alternative substrates for energy metabolism in adipocytes. It is noteworthy that Etomoxir reduced spare respiratory

capacity and maximal respiration in vehicle-treated cells but not in CDC1011-treated cells (Figure 4b and Supplementary Figure 6c), indicating that the oxidation capacity of control cells depends on the transport of fatty acids to mitochondria. The observed decrease in spare respiratory capacity in CDC1011-treated adipocytes, regardless of the presence of Etomoxir, suggests that CDC1011 induces fatty acid oxidation (FAO), which depletes spare respiratory capacity.

Additionally, We assess the impact of CDC1011 on glycolysis by measuring the glycolytic flux and glycolytic capacity in CDC1011-treated adipocytes and controls. Under basal conditions, the extracellular acidification rate (ECAR) revealed higher glucose consumption rates in CDC1011-treated adipocytes compared to controls. This difference became even more pronounced after glucose was added (Figure 4c). Furthermore, after inhibiting mitochondrial respiration using Rotenone and Antimycin A, CDC1011-treated adipocytes showed a greater increase in glycolytic flux than untreated adipocytes, indicating that CDC1011 treatment enhances glycolytic capacity to meet energy demands during metabolic stress (Figure 4c).

We also examined whether CDC1011 treatment affects cellular glucose uptake, a key characteristic of brown adipocytes. Basal and insulin-stimulated glucose uptake were measured in both undifferentiated and differentiated SW872 cells treated with CDC1011 or vehicle. CDC1011 treatment induced both basal and insulin-stimulated glucose uptake in both preadipocytes and mature adipocytes (Figure 4d). These results align with the observed CDC1011-induced thermogenic adipocyte-like phenotype, which relies on glucose consumption and glycolytic flux as crucial factors for maximizing thermogenesis in brown adipocytes.

Together, these findings suggest that CDC1011 enhances cellular metabolic flexibility by simultaneously increasing mitochondrial respiration and glycolytic flux, both of which are essential components of the thermogenic and energy-expending profile typical of brown adipocytes.

Transcriptomic profiling reveals mechanistic pathways

To characterize the transcriptional changes induced by CDC1011 during the early and late stages of SW872 maturation, we performed RNA sequencing. Principal component analysis

(PCA) of control and CDC1011-treated adipocytes at day 2 and day 7 post-differentiation revealed distinctive clustering of samples at both time points. This demonstrated minimal variation between replicates and a clear separation in transcriptional profiles between control and treated cells (Figure 5a).

Figure 5. Transcriptional profiling of CDC1011-treated adipocytes reveals upregulation of metabolic pathways and attenuation of inflammatory signaling. **a** Multi-dimensional scaling analysis of the transcriptional datasets of Control and CDC1011 (C10) treated adipocytes at day 2 and day 7. The variance explained by each dimension is indicated as a percentage in the axis titles. **b** Volcano plots showing differentially expressed genes from RNA sequencing of CDC1011 (C10) treated cells relative to control (CTRL) at day 2 (upper) and day 7 (lower). **c** GSEA analysis using hallmark gene set of the pathways that are reduced (highlighted in purple) and enhanced (highlighted in orange) in CDC1011-treated cells relative to control. **d** Heatmap of significantly regulated gene sets involved in different signaling represented as fold change difference of treated compared with the vehicle group at day 2 and day 7. ($P < 0.05$) are shown with significance next to each gene. p values were calculated by unpaired two-tailed Student's *t* test. *, P<0.05; **, adjusted P<0.05

Transcriptional analysis identified 2,775 upregulated genes and 2,385 downregulated genes in CDC1011-treated cells relative to controls at day 2, and 2,960 upregulated genes and 3,358 downregulated genes at day 7. Volcano plots confirmed the appropriate distribution of these differentially expressed genes, with many exhibiting greater than two-fold changes (FCs) (Figure 5b).

Gene Set Enrichment Analysis (GSEA) using the hallmark gene sets from the MSigDB database revealed that genes upregulated in CDC1011-treated cells were functionally enriched in pathways related to peroxisome and coagulation signaling, as well as metabolic processes including bile acid metabolism, glycolysis, fatty acid metabolism, and oxidative phosphorylation (Figure 5c and Supplementary Tables 11,12). These findings align with the observed CDC1011-induced activation of fatty acid oxidation, lipolysis, and glycolysis, as confirmed by cellular bioenergetic, lipolysis, and expression data. Conversely, genes downregulated in CDC1011-treated cells were associated with pathways involved in TNFα signaling, inflammatory response, interferon response, TGFβ signaling, adipogenesis, and IL-6 signaling (Figure 5c and Supplementary Tables 11 and 12, Supplementary Figures 7, 8).

Interestingly, many genes involved in inflammatory signaling were downregulated, while anti-inflammatory-related genes were enriched both early and late in differentiation upon treatment with CDC1011 (Figure 5d and Supplementary Figures 7,8). Additionally, p38 mitogen-activated protein kinase (p38 MAPK) signaling was predicted to be enriched, as major key regulators of this pathway were modulated upon treatment with CDC1011 (Figure 5d). p38 MAPK is one of the canonical thermogenic mechanisms activated downstream of the cAMP and PKA signaling axis, which leads to increased uncoupled respiration, lipolysis, and glucose utilization.

Some transcriptional changes were more evident later (at Day 7) during differentiation when treated with CDC1011. These changes included genes related to the respiratory electron chain (mitochondrion) (Figure 5d), cell cycle regulation, and chromatin organization (Supplementary Figure 9). The differential changes induced by CDC1011 early and late during differentiation might provide valuable insights into the primary molecular targets of this small molecule.

These findings suggest that CDC1011 not only activates thermogenic pathways but also alters key cellular processes, including metabolic and inflammatory responses, at various stages of adipocyte differentiation. The enriched pathways point to the molecule's broad potential to modulate energy metabolism and inflammatory signaling, making it a promising candidate for further exploration in metabolic disorders and thermogenesis-related therapies.

CDC1011 attenuates inflammation via cGMP and PDE5A

Based on our observation of a reduced inflammatory profile in brown adipocytes and our hypothesis that thermogenic inducers would mimic this effect, we further investigated the impact of CDC1011 on the inflammatory state in white adipocytes. Specifically, we measured the levels of pro-inflammatory factors in preadipocytes and differentiated SW872 cells treated with CDC1011 compared to vehicle. In both undifferentiated and differentiating adipocytes, we observed a significant reduction in the secretion of pro-inflammatory cytokines and obesity-associated factors, including IL-6, angiopoietin 1 and 2, BAFF, CXCL1, CXCL5, and osteopontin, upon treatment with CDC1011 (Figure 6a). Conversely, GDF-15 expression was markedly increased in both pre- and mature adipocytes treated with CDC1011 (Figure 6a). These findings suggest that CDC1011 not only induces a thermogenic phenotype but also has the potential to reduce the inflammatory response in adipocytes.

Figure 6. CDC1011 attenuates inflammation in human white adipocytes. **a** Cytokine array analysis of secreted factors in the supernatants from vehicle- and CDC1011-treated SW872 cells at both the preadipocyte and differentiated adipocyte stages. Downregulated cytokines are marked with red boxes and upregulated ones with green boxes. **b** Fold change in THP-1 cell migration towards conditioned media derived from pre-differentiated and differentiated SW872 treated with CDC1011 compared to vehicle. **c** Fold change in basal and forskolin-induced cGMP concentration measured by ELISA in CDC1011-treated preadipocyte and differentiating SW872 cells relative to control and normalized to the protein content. **d** and **e** Western blot analysis of (d) PDE5A and (e) phosphor-p65 (Ser536) expression measured in both preadipocytes and mature adipocytes treated with CDC1011 or DMSO. ANG1, Angiopoietin 1; OPN, Osteopontin; GDF15, Growth/differentiation factor 15; BAFF, B-cell activating factor; CXCL5, C-X-C Motif

Chemokine Ligand 5; CXCL1, C-X-C Motif Chemokine Ligand 1; IL6, Interleukin 6. Presented results are representative of biological replicates (N=3). p values were calculated by unpaired two-tailed Student's *t* test. *, P<0.05; **, P<0.01; ***, P<0.001

We also evaluated the chemotaxis of monocytes toward chemokines secreted by CDC1011 and vehicle-treated pre- and mature adipocytes. The migration of THP-1 cells was significantly reduced toward the supernatant derived from both pre- and mature adipocytes treated with CDC1011 (Figure 6b). This further supports the anti-inflammatory effect of CDC1011, as it reduces the secretion of pro-inflammatory factors that drive monocyte migration.

One interesting observation from the transcriptomic data and IPA prediction was the enrichment of gene sets involved in the PDE5A inhibition pathway and nitric oxide (NO) signaling. PDE5A is a cyclic guanosine monophosphate (cGMP)-selective phosphodiesterase that regulates cGMP signaling by degrading cGMP to GMP [67]. cGMP is involved in the regulation of adipose tissue [68-71] function through cGMP-dependent protein kinase (PKG) [68,69]. cGMP-PKG signaling promotes thermogenesis, lipolysis, glucose uptake, healthy adipose tissue expansion, and adipokine secretion in white adipocytes [68,69,71-74]. Given the role of cGMP signaling in regulating metabolic homeostasis and proinflammatory responses in adipose tissue, we measured the cGMP levels in adipocytes treated with CDC1011. CDC1011 treatment significantly increased both basal and forskolin-induced cGMP levels in both preadipocytes and adipocytes (Figure 6c). Furthermore, western blot analysis showed a substantial reduction in PDE5A, a cGMP-specific phosphodiesterase, in CDC1011-treated preadipocytes and mature adipocytes (Figure 6d). These data suggest that CDC1011 promotes thermogenesis and anti-inflammatory effects in adipocytes at least in part by reducing PDE5A expression and consequently increasing cGMP availability.

One of the PKG reported substrates is the NF-κB subunit p65. NF-κB signaling is a major mediator of inflammation associated with obesity, and our transcriptomic data predicted that this pathway would be reduced upon CDC1011 treatment. Therefore, we checked the phosphorylation of the NF-κB subunit p65 at serine 536 (Ser536), as it has been shown to inhibit NF-κB signaling and prevent inflammation [75,76]. Our data showed that CDC1011 treatment induces the phosphorylation of p65 at serine 536 (Figure 6e), suggesting that CDC1011 may mitigate inflammation by enhancing the phosphorylation of NF-κB, leading to its inhibition.

Taken together, these findings provide compelling evidence that CDC1011 not only promotes thermogenesis and metabolic flexibility but also exhibits anti-inflammatory properties in adipocytes. These effects, mediated through the modulation of cGMP-PKG signaling and NF-κB inhibition, make CDC1011 a promising candidate for future therapeutic interventions aimed at treating metabolic diseases associated with obesity and chronic inflammation.

Identifying HDACs as key targets of CDC1011

To identify molecular target(s) of CDC1011, we combined computational prediction with experimental validation. Transcriptomic data from CDC1011 treated SW872 cells revealed differential gene expression patterns consistent with chromatin remodeling and transcriptional regulation (Supplementary Figure 9). Upstream regulator prediction by IPA of CDC1011 treated cells transcriptomic data (Supplementary Tables 13,14) converged on HDACs, including HDAC2 and HDAC3, as top common candidate targets. Also, HDAC inhibitors (trichostatin A, dacinostat, Panobinostat and vorinostat) emerged among the top candidate drugs in IPA prediction (Supplementary Tables 13,14). The presence of tetrazole ring in CDC1011, a heterocyclic moiety used in potent and selective HDAC inhibitors [77], further supported the selection of HDAC as a as potential mediators of the CDC1011's biological activity potential candidate.

To verify and further characterize the interaction between CDC1011 and HDACs, we evaluated the binding affinity and isoform selectivity trend of CDC1011 against a panel of HDAC proteins through molecular docking simulations. Docking poses were ranked by

Rosetta total score and interface energy (I_sc), with lower scores indicating more favorable binding. Our comparative docking analysis revealed distinct binding profiles across HDAC isoforms. Notably, all tested HDACs yielded moderate productive-decoy rates and tightly overlapping Min Interface Δ_int values, showing only subtle enantiomeric biases (R favored on HDAC2, HDAC3, HDAC8; S on HDAC6, HDAC11 and HDAC1). Total-score minima further discriminate performance where HDAC6 and HDAC11 exhibit the deepest well, whereas HDAC8 has the shallowest. These results strongly support the hypothesis that one or more HDACs serve as direct binding target (s) of CDC1011.

Scorpion interaction analysis ranks tight binding interactions, highlighting hotspots where tight binding network result in high affinity between CDC1011 and HDACs pockets as can be visualized in Figure 7a, -b and Supplementary Figure 10).

To confirm the predicted interaction between CDC1011 and HDACs, we conducted a biochemical HDAC activity assay using crude HDACs from nuclear extracts. CDC1011 treatment resulted in a significant 50% reduction in total HDAC activity compared to vehicle controls (Figure 7c). These findings strongly suggest that CDC1011 functions as an HDAC inhibitor, potentially through direct binding or interference with upstream regulatory complexes (Figure 8).

Figure 7. CDC1011 targets HDACs. A Multi-panel comparison of Rosetta total docking score versus interface Δ (ΔI) for the R (top row) and S (bottom row) enantiomers across six HDAC isoforms (HDAC1, HDAC2, HDAC3, HDAC6, HDAC8, HDAC11). Each gray circle represents an individual docking pose; green circles highlight the ten poses with the most favorable (lowest) ΔI values, and the red circle marks the single pose with the global minimum ΔI for each enantiomer. **b** Representative binding poses of the R-enantiomer CDC1011 across the

HDAC 2, 3, 6 and 11. Global views of CDC1011 (orange sticks) bound to HDAC2 (cyan surface), HDAC3 (lavender surface), HDAC6 (gray surface) and HDAC11 (ochre surface). **c** HDAC activity as a percentage of control upon incubation of crude HDACs with CDC1011 (5μM) and a positive control TSA (1μM). p values were calculated by unpaired two-tailed Student's *t* test. *, P<0.05; **, P<0.01; ***, P<0.001

Figure 8. Diagram summarizing the changes induced by CDC1011 in adipocytes. Dashed lines indicate a reduction upon CDC1011 treatment and undashed lines indicate an induction upon CDC1011 treatment. PDE, Phosphodiesterase; TG, Triglycerides; FA, Fatty acids; GLUT, Glucose transporter. Created in https://BioRender.com

Discussion and Conclusions

Obesity drives a systemic proinflammatory state, with serum from obese individuals enriched in TNF-α, IL-1β, IL-6, IL-12, IP-10, MIP-1β, and MCP-1. This profile is mirrored during human white adipocyte differentiation *in vitro*, where mature white adipocytes secrete higher chemokine levels than brown adipocytes, promoting monocyte recruitment and M1-like macrophage polarization. Motivated by these insights, we conducted a high-throughput screen in human white adipocytes to identify small molecules that simultaneously induce browning and suppress inflammation. This effort yielded CDC1011,

a novel compound that robustly enhances thermogenesis, substrate oxidation, lipolysis, and glucose uptake while reprogramming the adipocyte secretome toward an anti-inflammatory profile. These dual effects establish proof-of-concept for integrated therapeutic agents targeting obesity's core pathologies.

Elevated adipose inflammation is a hallmark of obesity [78-83], with WAT exhibiting greater macrophage infiltration and cytokine production than BAT in humans and mice [84]. Brown adipocytes intrinsically dampen inflammatory signaling, rendering thermogenic activation an attractive anti-obesogenic strategy [85]. CDC1011 induces browning in pre- and mature white adipocytes, upregulating β3-adrenergic receptor (ADRB3), brown/beige markers, and mitochondrial electron transport chain subunits. Functional consequences include accelerated lipolysis, glucose uptake, metabolic flexibility, and a shift from proinflammatory to anti-inflammatory cytokine profiles. Transcriptomic profiling revealed enrichment of glycolysis, fatty acid oxidation, and oxidative phosphorylation pathways, consistent with the metabolic reprogramming of beige adipocytes [86-92].

CDC1011 boosts mitochondrial respiration using both glucose and fatty acids mirroring the high substrate flux of brown adipocytes. Upregulation of glucose transporters (SLC2A1, SLC2A3, SLC2A4, SLC2A6) supports enhanced glycolytic capacity and may contribute to improved insulin sensitivity. The observed increase in lipolysis can be explained by the induction of LIPE (hormone-sensitive lipase) and ADRB3 signaling. This is consistent with the elevated cAMP levels, which reflect β3AR/PKA activation. The sustained cAMP signaling is likely due to the downregulation of PDE4D [93-95], whereas PDE5A suppression elevates cGMP, which in turn engages PKG-dependent lipolysis and glucose homeostasis [96]. Inhibition of PDE4/5 isoforms also likely accounts for the anti-inflammatory effects, as PDE inhibitors are known to potently suppress cytokine release in adipocytes and macrophages [97-102].

Our gene set enrichment analysis showed suppression of TNF-α/NF-κB signaling, unfolded protein response, and interferon pathways known as core nodes of obesity-driven inflammation [103]. Cytokine profiling confirmed reduced IL-6 and induction of anti-

inflammatory mediators GDF15, IL-33, and TGF-β1. GDF15, a thermogenesis-responsive adipokine, directly inhibits macrophage activation [104,105], linking CDC1011-induced browning to paracrine immune modulation.

CDC1011 inhibits HDAC activity in adipocyte lysates, with docking revealing preferential binding to class I (HDAC3) and class IV (HDAC11) isoforms, which are established critical regulators of adipocyte metabolism and thermogenesis [106]. Unlike SIRT1 of Class III HDAC [107-109], Class I HDAC inhibition drives UCP1 and PGC1-α expression [110], whereas HDAC11 ablation enhances β-adrenergic signaling, oxygen consumption, and obesity resistance [111-114] through BRD2 and gravin-α interactions [115]. The isoform-specific docking signatures of CDC1011 predict selective HDAC modulation, a critical advantage for minimizing off-target effects. These data position HDAC inhibition as a central node in CDC1011's mechanism, with thermogenic and anti-inflammatory outcomes converging on epigenetic control of browning and NF-κB suppression.

RNA-seq also highlighted TRPM8 and voltage-gated calcium channels (CACNA1G, CACNA2D2, CACNG6, CACNG8), implicating calcium flux in CDC1011's thermogenic program. The compound's scaffold and polypharmacology profile suggest additional targets, to be mapped by thermal proteome profiling and chemoproteomics in follow-up studies.

By integrating browning, catabolic flux, and inflammation resolution, CDC1011 outperforms single-pathway interventions and positions HDAC/PDE modulation as a viable axis for obesity therapeutics. This human-centric approach highlights translational potential, as reprogramming WAT into metabolically active, immune-tolerant tissue could ameliorate insulin resistance and type 2 diabetes in patients.

As with many preclinical studies, our work relies on *in vitro* human adipocyte models that offer key mechanistic insights.While *in vivo* validation is planned (e.g., diet-induced obesity mouse models for pharmacokinetics, efficacy, and safety), this human-centric study provides proof-of-concept for dual thermogenic/anti-inflammatory effects in obesity. Target deconvolution via thermal proteome profiling and chemoproteomics will further

map CDC1011's interactome, supporting lead optimization toward clinical candidates for obesity and related comorbidities.

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Author Contribution

LC and JS conceptualized the study. AG and JS characterized the proinflammatory status in obesity using human samples and cell lines. AD made the MCR library, and JS developed the

luciferase-based screening platform. AAR and SA conducted the drug screening, while MR designed and performed experiments, analyzed data, and characterized CDC1011. NH carried out bioinformatics analysis. MR, AAR, and JS contributed to manuscript writing. All authors participated in reviewing and editing the drafts. The corresponding author, LC, had the ultimate responsibility for manuscript submission.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions. RNA-seq raw and processed data will be deposited in GEO (accession to be provided upon acceptance or prior to revision). Materials (CDC1011 and selected MCR compounds) are available for academic use under an MTA.

Ethical approval

Human plasma sampling and monocyte isolation were approved by the Hamad Medical Corporation Research Ethics Committee and the Weill Cornell Medicine-Qatar IRBcommittee. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Conflicts of interests

The authors declare no potential conflicts of interest.

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