

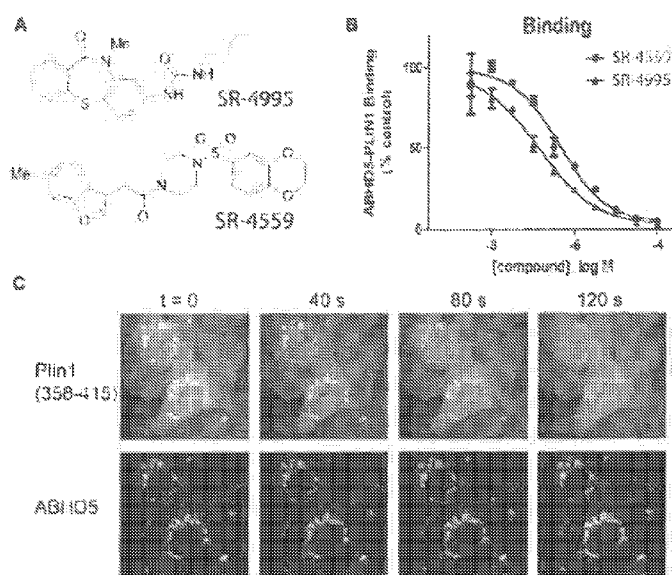


- (51) International Patent Classification:
A61K 36/82 (2006.01) *A61P 3/04* (2006.01)
- (21) International Application Number:
PCT/US2016/053005
- (22) International Filing Date:
22 September 2016 (22.09.2016)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
62/222,323 23 September 2015 (23.09.2015) US
- (71) Applicants: **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US). **WAYNE STATE UNIVERSITY** [US/US]; 656 West Kirby, Detroit, MI 48202 (US).
- (72) Inventors: **ROUSH, William, R.**; 1013 Town Hall Avenue, Jupiter, FL 33458 (US). **GRANNEMAN, James, G.**; 9109 Yorkshire Drive, Saline, MI 48176 (US). **HALVORSEN, Geoff, T.**; 29625 N. Waukegan Road, #206, Lake Bluff, IL 60044 (US).
- (74) Agents: **FITTING, Thomas** et al.; The Scripps Research Institute, 10550 North Torrey Pines Road, TPC-8, La Jolla, CA 92037 (US).
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: SMALL MOLECULE MODULATORS OF CELLULAR LIPOLYSIS

Figure 1



(57) Abstract: The invention provides a compound which binds ABHD5, disrupts complexes of ABHD5 and PLIN1 or PLIN5, and stimulates cellular lipolysis. The compound can be used, in an effective dose, to promote fat catabolism. Consequently, the invention also provides a method of treatment of obesity, diabetes, cardiovascular disease, or cancer when an effective amount of the compound is administered to a patient. The invention further provides a method of treatment of ichthyosis and a method of increasing the content in skin of skin barrier lipids, comprising applying an effective amount or concentration of the compound topically.

WO 2017/053510 A1



Published:

— *with international search report (Art. 21(3))*

Small Molecule Modulators of Cellular Lipolysis

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the priority of U.S. provisional application serial number 62/222,323, filed Sept. 23, 2015, the disclosure of which is incorporated herein in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under MH084512, DK62292, DK76629, DK091741, and NS21061634 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

The storage and mobilization of lipid energy are fundamental processes of virtually all eukaryotic cells. It is well known that chronic mobilization of fatty acids in brown and white adipocytes by agents that elevate protein kinase A (PKA) activity has potent anti-obesity effects in animal models.¹⁻³ In fat cells, the mobilization of fatty acids from stored triacylglycerol is thought to involve the functional interactions of three highly conserved proteins: adipose triglyceride lipase (ATGL), α - β hydrolase-containing protein 5 (ABHD5), and perilipin-1 (PLIN1).^{4,5} ABHD5 is a potent activator of ATGL;⁶ however, this lipase-promoting activity is suppressed under basal conditions when ABHD5 is bound to PLIN1 on the surface of lipid droplets.⁷ Extracellular signals that lead to the phosphorylation of PLIN1 by protein kinase A (PKA) release ABHD5, which activates ATGL, the rate limiting step in lipolysis. Presently, PKA-mediated phosphorylation is the only known mechanism for the rapid (seconds to minutes) mobilization of lipid energy from adipocytes.

The storage and mobilization of lipid energy are fundamental processes of virtually all eukaryotic cells. Mobilization of fatty acids from stored triacylglycerol is thought to occur on the surface of intracellular lipid droplets (LD), and recent work indicates that lipolysis is controlled by the functional interactions of three highly conserved proteins: perilipin (PLIN), α - β hydrolase-containing protein 5 (ABHD5), and adipose triglyceride lipase (ATGL).^{4,5} ATGL is responsible for the first and rate limiting step of triacylglycerol hydrolysis in adipocytes, skeletal muscle, and heart. ABHD5 (also known as CGI-58) is a critical upstream regulator of ATGL that potently increases the lipase activity of ATGL and possibly other lipases.⁶ Importantly, human mutations of ATGL and ABHD5 lead to an overlapping spectrum of disease involving ectopic neutral lipid accumulation in several tissues.¹⁰⁻¹³

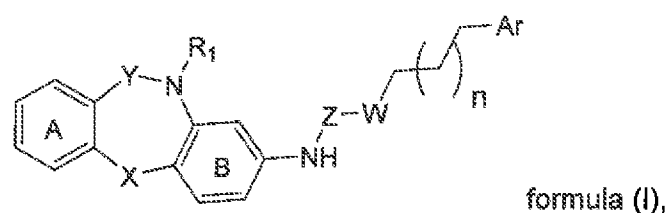
ABHD5 has also been implicated in intracellular lipolysis in tissues that lack PLIN1, such as skeletal muscle and heart, where PLIN5 binds ABHD5 and suppresses basal and stimulated lipolysis.¹⁴⁻¹⁶ Interestingly, the interaction of PLIN5 and ABHD5 is not affected by PKA-mediated phosphorylation,¹⁴ suggesting that ABHD5 might regulate lipolysis independently of PKA in other cell types.

SUMMARY

Here we identify novel synthetic ligands that disrupt complexes of ABHD5 and PLIN1 or PLIN5, and rapidly active lipolysis in adipocytes and muscle in the absence of PKA activation. We found that activating ligands directly bind to ABHD5 within a highly conserved substrate-binding motif near the c-terminus. This work demonstrates that ABHD5 is a ligand-regulated lipase co-activator that lies downstream of extracellular signaling pathways. We propose that ABHD5 ligands might be developed into a novel class of therapeutics that more directly promotes fat catabolism⁸ and overcomes limitations associated with extracellular signaling pathways.⁹

Drugs that stimulate lipid breakdown in adipocytes and muscle currently rely on the activation of transmembrane signaling. The compounds we have identified bypass transmembrane signaling and activate lipolysis directly. The goal is to develop clinically useful agents targeting lipid disorders such as obesity, diabetes, and cardiovascular disease.

Accordingly, the invention provides, in various embodiments, a method of stimulating cellular lipolysis by disrupting complexes of PLIN1-ABHD5 or PLIN5-ABHD5, comprising contacting ABHD5 with an effective amount or concentration of a compound wherein the compound is of formula (I)



wherein

ring A and ring B are each independently aryl, 5-membered heteroaryl, or 6-membered heteroaryl;

wherein ring A and ring B each optionally bear 1-3 independently selected V, where each V is independently at each occurrence halogen, cyano, nitro, CF₃, lower alkyl, lower alkoxy, CO₂H, CO₂R, or CONR₂;

X and W are each independently S, O, NR or CR, wherein R is H, alkyl or aryl;

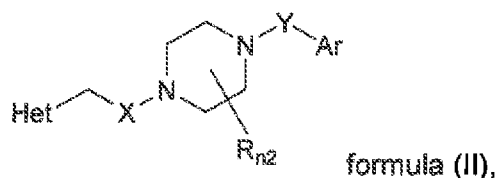
Y and Z are each independently CO or SO₂;

Ar is substituted aryl or heteroaryl;

n = 0-5;

or a pharmaceutically acceptable salt thereof;

or wherein the compound is of formula (II)



wherein

Het is a 5-membered heteroaryl, a 6-membered heteroaryl, or a bicyclic heteroaryl;

Ar is aryl or heteroaryl;

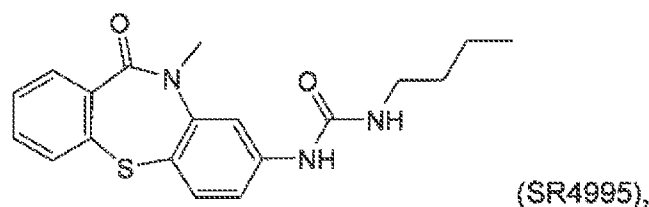
wherein Het and Ar each optionally bear 1-3 independently selected V, where each V is independently at each occurrence halogen, cyano, nitro, CF₃, lower alkyl, lower alkoxy, CO₂H, CO₂R, or CONR₂;

X and Y are each independently CO or SO₂;

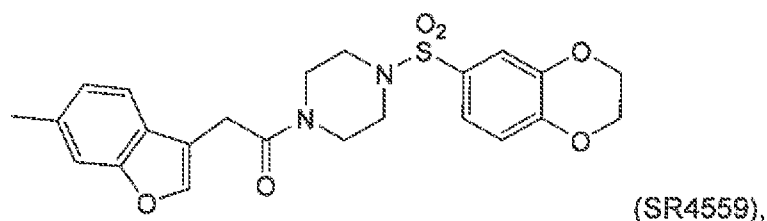
or a pharmaceutically acceptable salt thereof.

The invention further provides a compound of formula (I) or of formula (II). In various embodiments, the compound is not SR4995 or SR4559.

In various embodiments of a method of the invention, the compound is



or



or a pharmaceutically acceptable salt thereof.

In various embodiments of a method of the invention, the compound is any one of the compounds shown in Table 1, below.

The invention further provides a method of promoting fat catabolism, comprising administering to a patient an effective dose of a compound of formula (I) or formula (II). Consequently, in various embodiments, the invention can provide a method of treatment of obesity, diabetes, cardiovascular disease, or cancer, comprising administering to a patient afflicted therewith an effective dose of a compound of formula (I) or formula (II).

The invention further provides a method of treatment of ichthyosis, comprising applying to the skin of a patient afflicted therewith an effective amount of a topical formulation comprising a compound of formula (I) or formula (II), and a method of increasing the content in skin of skin barrier lipids, comprising applying to skin an effective amount of a topical formulation comprising a compound of formula (I) or formula (II).

The invention further provides a compound of formula (I) or of formula (II). In various embodiments, the compound is not SR4995 or SR4559. In various embodiments, the invention provides a compound of formula (I) or formula (II) for disrupting a PLIN1-ABHD5 complex to stimulate adipocyte lipolysis, for promoting fat catabolism, for treatment of ichthyosis, and for increasing the content in skin of skin barrier lipids.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. HTS identification of compounds that disrupt the interaction of PLIN1 with ABHD5. A: Structure of the tricyclic thio urea (TTU) SR-4995 (Compound 34), and the sulfonyl piperazine (SPZ) SR-4559 (Compound 44). B: Compounds SR-4995 and SR-4559 block the interaction of PLIN1 with ABHD5 in the luciferase complementation assay (Binding) and stimulate lipolysis in cultured brown adipocytes nearly as effectively as maximal stimulation by 10 μ M isoproterenol. C: Compound SR-4995 dissociates EYFP-PLIN1 from lipid droplet-targeted ECFP-ABHD5 (red pseudo-color) in live brown adipocytes within 2 minutes.

Figure 2. TTU and SPZ ligands activate brown adipocyte lipolysis potently and independently of PKA-signaling A: Compounds SR-4995 (Compound 34) and SR-4559 (Compound 44) stimulate lipolysis with EC_{50} of 4-7 μ M and maximal effect is > 75% of the maximal effect produced by isoproterenol (10 μ M). B: Compound SR-4559 rapidly stimulates lipolysis, indicated by fluorescence increase on lipid droplets (arrows), in live brown adipocytes. Lipolysis is observed within 4 minutes of cellular application. Details of this imaging assay are published in.¹⁸ Although not shown, both compounds are active in each assay. C: Stimulation of lipolysis by compounds SR-4995 and SR-4559 is independent of PKA-mediated phosphorylation. Brown adipocytes were treated with isoproterenol (10 nM) or compounds SR-4995 and SR-4559 (10 μ M) for 30 minutes before determining fatty acid release and

phosphorylation status of hormone-sensitive lipase (HSL). Left: Agents produced similar levels of lipolysis. Right: isoproterenol treatment triggered strong phosphorylation of HSL on established PKA target sites, whereas compounds SR-4995 and SR-4559 were without effect.

Figure 3. Stimulation of lipolysis by compounds SR-4995 (Compound 34) and SR-4559 (Compound 44) depends on ABHD5 and ATGL. A: Compounds SR-4995 and SR-4559 (10 μ M) stimulate lipolysis in wild type (WT) brown adipocytes and stable knockdown of ABHD5 by shRNA (insert) eliminates compound efficacy. B: Rescue of ABHD5 expression reinstates compound efficacy. The brown adipocyte ABHD5 knockdown cell line was transduced with a lentiviral vector that allowed doxycycline (Dox) induction of ABHD5 that is resistant to shRNA (insert). In the absence of Dox, compounds SR-4995 and SR-4559 are inactive and the efficacy of isoproterenol (Iso) is severely reduced. Expression of ABHD5 by doxycycline (Dox) reinstates the efficacy of compounds SR-4995 and SR-4559, and increases responsiveness to isoproterenol by 5-fold. C: Compounds SR-4995 and SR-4559 stimulate lipolysis in isolated white adipocytes, and this effect is abolished by adipocyte-specific knockout of ATGL. D: Compounds do not augment the ability of ABHD5 to stimulate lipase activity of ATGL using artificial substrates in vitro.

Figure 4. NBD affinity ligand identifies ABHD5 as the target of compounds SR-4995 and SR-4559. A: Cos7 cells expressing ABHD5 were labeled with NBD (50 μ M) in DMSO or compounds SR-4995 or SR-4559 (50 μ M) for indicate times. Cellular proteins were resolved by SDS PAGE and labeled proteins imaged by laser-scanning fluorometry. Compounds SR-4995 and SR-4559 blocked NBD labeling of ABHD5. B: NBD labels both HSL and ABHD5 in transfected Cos7 cells, and compound SR-4995 potentially blocks labeling of ABHD5, but not HSL. C: Mass spectrometry of NBD-labeled ABHD5 identifies Y330 as the amino acid covalently modified by the affinity ligand. D: Mutation of Y330 to alanine (A) or phenylalanine (F) eliminates covalent modification of ABHD5 by NBD. Non, nontransfected cells.

DETAILED DESCRIPTION

Based on the fact that binding of PLIN1 to ABHD5 potentially inhibits its ability to activate ATGL, we hypothesized that agents which disrupt PLIN1-ABHD5 complexes would stimulate adipocyte lipolysis.⁷ To find such agents, we devised an assay for the interaction of ABHD5 with PLIN1 or PLIN5 using luciferase complementation.^{7,14,17} In this assay, inactive N- and C-terminal fragments of luciferase were fused to PLIN1, PLIN5 or ABHD5, respectively, and the binding reaction, which occurs in cell-free lysates, was reported by the restoration of luciferase activity (PubChem AID 453907, AID 651674). The primary screen monitored the ability of

compounds to suppress the interaction of ABHD5 with PLIN5. Hits from this screen were then evaluated for non-specific inhibition of a control protein-protein interaction (formation of hepatic nuclear factor-4 (HNF4) dimers), as well as inhibition of ABHD5-PLIN1 luciferase complementation. Screening of >364,000 compounds from the NIH chemical library identified 5 compounds, representing 3 independent chemical scaffolds, that inhibited the interaction of ABHD5 with PLIN1 and PLIN5, and stimulated lipolysis in cultured brown adipocytes.

Shown in Figure 1A are representative members of two scaffolds, thiaza-tricyclo-ureas (TTU) represented by SR-4995 (Compound 34 herein), and sulfonyl piperazines (SPZ) represented by SR-4559 (Compound 44 herein). These compounds prevented binding of ABHD5 to PLIN1 in luciferase complementation assays with IC_{50} s <1 μ M (Figure 1B). The high throughput protein complementation assays were performed in cell-free lysates, and luminescence is an indirect read-out of protein-protein interaction. To verify that compounds disrupt the direct interaction of ABHD5 with PLIN1 in live cells, we identified a minimal fragment of human PLIN1 (amino acids 358-417) that binds ABHD5 in Cos7 cells. Importantly, this PLIN1 fragment does not bind lipid droplets in the absence of ABHD5, so the effect of compounds can be readily observed as the release of the EYFP-tagged PLIN1 fragment from LD containing ECFP-tagged ABHD5. In the resting state, PLIN1 and ABHD5 were colocalized on lipid droplet surfaces. Compound SR-4559 (1 μ M final) triggered the release of the PLIN1 fragment from ABHD5 within 1 minute of extracellular application (Figure 1C).

We next tested the effects of compounds on lipolysis in cultured brown adipocytes. We found that compounds SR-4995 and SR-4559 stimulated lipolysis with EC_{50} s of about 4 μ M, and that maximally-effective concentrations produced lipolytic rates that were > 75% of that produced by isoproterenol, a full beta receptor agonist. We examined the temporal relation between compound application and intracellular fatty acid mobilization in live cells by fluorescence microscopy. In this assay, mobilized fatty acid trigger that translocation of EYFP-tagged SRC1 to lipid droplets to where the ligand binding domain of PPAR α has been targeted (Figure 2B).^{16,19} We found that compounds rapidly trigger lipolysis (within 3 minutes) on a timeframe that is similar to the dissociation of PLIN1-ABHD5 complexes. As mentioned above, activation of cAMP-dependent PKA is the only known mechanism for rapid activation of adipocyte lipolysis. As expected, stimulation of lipolysis by the β -adrenergic agonist isoproterenol was associated with strong phosphorylation of hormone sensitive lipase (HSL) on established PKA sites, whereas equally-effective concentrations of reference compounds SR-4995 and SR-4559 triggered lipolysis in the absence of phosphorylation activation (Figure 2C).

To verify the mechanism of action, we performed knockdown and rescue experiments with ABHD5. As shown in Figure 3A and B, TTU and SPZ compounds (10 μ M) increased lipolysis by ~7 fold in cultured brown adipocytes, and stable knockdown of ABHD5 by lentiviral shRNA (insert) abolished the ability of compounds to stimulate lipolysis. We transduced the knockdown cell line with a doxycycline-inducible ABHD5 construct that is resistant to the viral shRNA (Figure 3B). When tested in knockdown adipocytes, compounds were completely inactive in the absence of doxycycline, and the activity induced by isoproterenol was also reduced by ~85%. Reinstatement of ABHD5 expression with doxycycline completely restored the efficacy of the TTU and SPZ agonists as well as isoproterenol, establishing that stimulation of lipolysis by compounds requires ABHD5. Compounds SR-4995 and SR-4559 stimulated lipolysis in freshly-isolated mouse white adipocytes, and this effect was eliminated by adipocyte-specific knockout of ATGL (Figure 3C), the lipase target of ABHD5. We confirmed that ABHD5 potently increases the activity of ATGL against artificial lipid droplets *in vitro* (Figure 3D), and found that SR-4995 and SR-4559 did not modify this effect. These observations indicate that the compounds mainly act by relieving inhibition by PLIN1, and that binding of compounds *per se* does not affect the ability of ABHD5 to activate ATGL.

In theory, compounds that dissociate complexes could bind either ABHD5 or PLIN1, or perhaps an interface created by the interaction of these proteins. As mentioned above, ABHD5 binds to PLIN1 and PLIN5, and we found that compounds were similarly potent in disrupting the interaction of ABHD5 with PLIN1 or PLIN5 suggesting that ABHD5 was the likely target. To determine whether ABHD5 is the direct binding target, we made use of an affinity probe, NBD-HE-HP (NBD) that was previously shown to covalently modify ABHD5.²⁰ As shown in Figure 4A, the NBD-HE-HP (NBD) affinity tag covalently modified ABHD5 in transfected Cos7 cells, and this labeling was blocked by co-incubation with TTU and SPZ ligands (50 μ M). Importantly, SR-4995 blocked NBD binding to ABHD5 with a potency nearly identical to that observed for disrupting the interaction of ABHD5 with PLIN1 (Figure 4B). As expected,²⁰ the NBD affinity probe also labeled HSL expressed in transfected COS7 cells; however, SR-4995 did not block NBD binding to HSL, demonstrating the specificity of this ligand for binding ABHD5 (Figure 4B).

NBD was developed as an active site affinity label of serine hydrolases; however, because ABHD5 lacks the conserved catalytic serine seen in related α - β hydrolases,^{10,13,21} we reasoned that the site of modification and ligand interactions likely involves a novel binding domain. To determine the site of protein modification, we subjected purified NBD-labeled and unlabeled ABHD5 to LC/MS/MS analysis and observed 10 unique spectra demonstrating that

Y330 is modified by NBD. Shown in Figure 4C are representative MS-2 spectra of peptide Thr321-Lys342 from unmodified (top) and NBD-labeled ABHD5 (bottom). The m/z difference of b_9 and b_{10} ions in unlabeled and labeled ABHD5 unambiguously identified Y330 as the site of NBD modification, which was confirmed by the mass shift of the remaining b ions. As expected, mutation of Y330 to alanine or phenylalanine prevented tagging by NBD (Figure 4D). The labeling of Y330 in ABHD5 by the serine hydrolase activity probe likely represents an interaction at a promiscuous substrate-like binding site.²² In this regard, Y330 lies within the highly conserved sequence HYVYAD sequence of ABHD5 that resembles the H4XD (where X is any amino acid) motif observed in numerous hydrolases and acyl-transferases.^{23,24}

The mechanism by which ABHD5 activates lipolysis has not been established, but is thought to involve allosteric interactions with ATGL.^{6,26} ABHD5 has no established enzymatic activity²⁶ and, as mentioned above, the nucleophilic serine found in closely-related α - β hydrolases is a catalytically-inactive asparagine in ABHD5. Interestingly, the asparagine substitution is conserved in chordates, suggesting that the ligand/substrate binding pocket, but not enzymatic activity, may be functionally important for ABHD5.

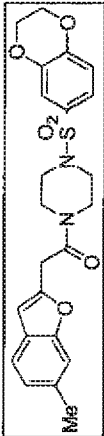
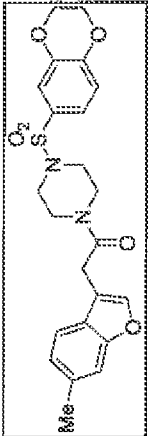
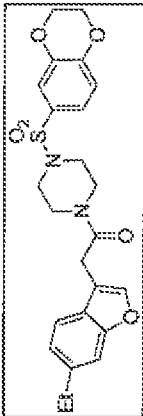
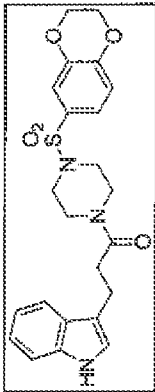
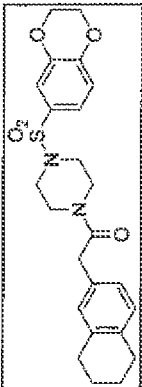
Our current understanding indicates that rapid control of lipolysis involves the integration of extracellular stimulatory and inhibitory signals by PKA, which phosphorylates the key lipolytic effectors PLIN1 and HSL.^{7,27,28} The demonstration that ABHD5 is sensitive to activating ligands exposes a new regulation of lipolysis that bypasses the PKA signaling pathway, and raises several important questions. Firstly, do ABHD5 synthetic ligands exploit binding sites used by endogenous ligands? Most of our understanding of intracellular lipolysis comes from analysis of adipocytes whereby extracellular signals, such as catecholamines, mobilize fatty acids for export and systemic oxidation. Numerous tissues, such as contracting muscle, mobilize fatty acids from intracellular lipid droplets for oxidation *in situ*; however, the mechanisms that link energy demand to internal supply are poorly understood. We note that PLIN5 is the major ABHD5 binding protein of heart and oxidative muscle,^{14,16,29} and speculate that these complexes are well positioned to respond to endogenous ligands that might couple internal demand to fatty acid supply. Secondly, might ABHD5 ligands be developed into novel therapeutic entities? It is well-established that persistent activation of adipocyte lipolysis by cold stress or β 3 adrenergic receptors has potent anti-obesity and anti-diabetes properties in rodent models.⁹ Under these conditions, mobilized fatty acids upregulate fat oxidation and thermogenesis within brown and white adipose tissues, resulting in lower circulating fatty acids, reduced adiposity, and improved insulin action.^{1,9} Given that fatty acids are necessary and sufficient for brown adipocyte

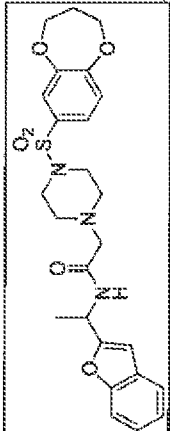
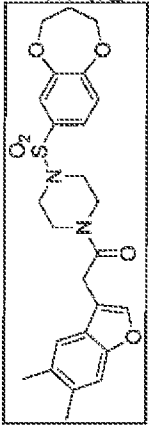
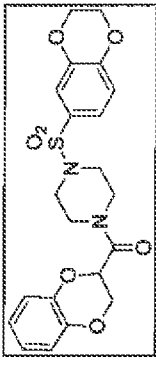
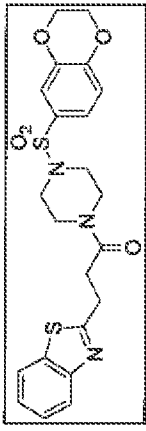
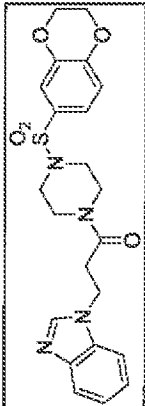
thermogenesis³⁰⁻³² and adipose tissue-specific overexpression of ATGL improves metabolic status of obese mice,⁹ ABHD5 ligands might provide a more direct means of promoting fat catabolism in numerous tissues that overcomes limitations associated with surface receptor expression.

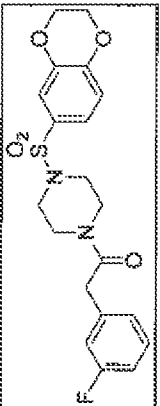
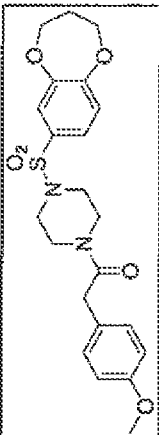
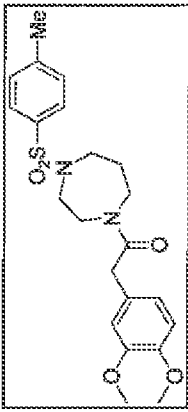
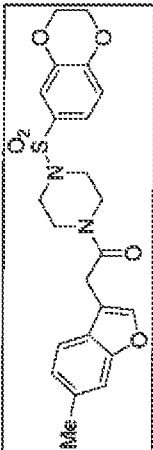
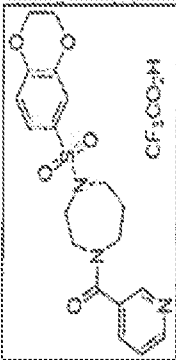
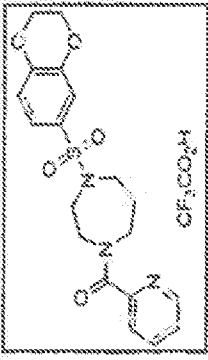
In summary, we have discovered a new molecular pharmacology of lipolysis that involves ligand-mediated activation of ABHD5, a critical lipase-activating protein. Given the high degree of conservation in the ligand binding domain of ABHD5, we hypothesize that the probes we have identified mimic endogenous ligands and suggest a new site of lipolytic regulation. Moreover, we anticipate that development of these novel chemical scaffolds may lead to modulators of tissue lipolysis that might be useful in the treatment of metabolic disease.

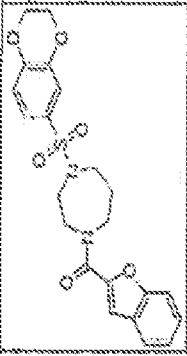
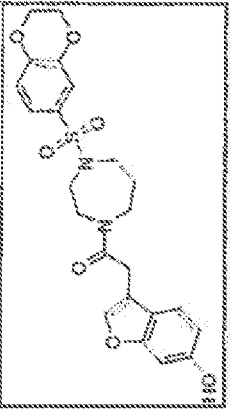
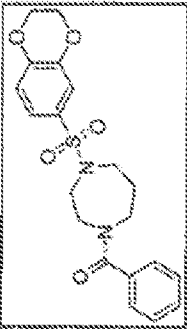
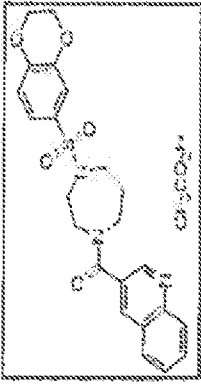
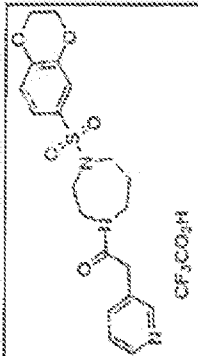
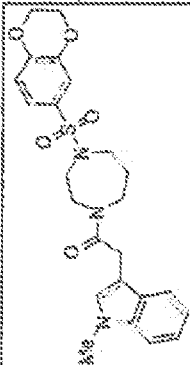
Table 1 provides a summary of compounds and the corresponding bioactivities of selected examples. PCA refers to Protein fragment Complementation Assay, which measures disruption of the PLIN5-ABHD5 interaction. NA = not active. ISO = isoproterenol.

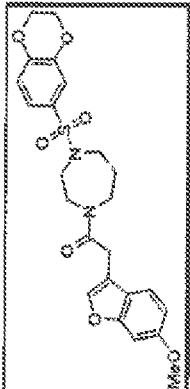
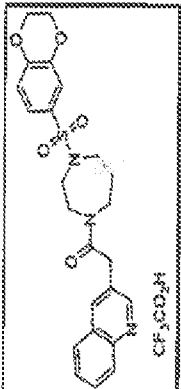
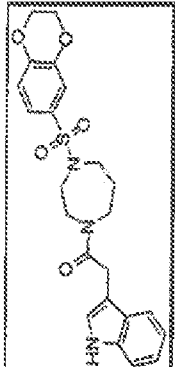
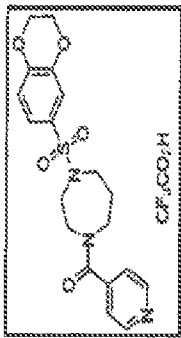
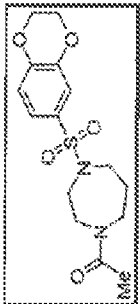
Table 1: Structures and Bioactivity Data

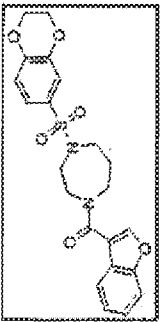
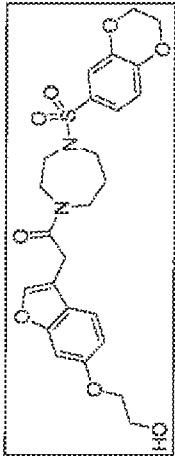
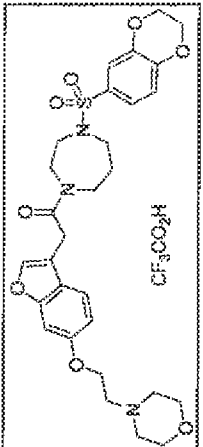
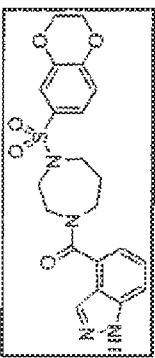
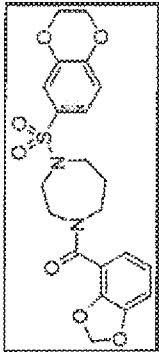
Structures	SR numbers (03-00000XXXX)	BA Lipolysis		PCA	
		EC50	% of ISO	IC50	IC50 for 1055
	SR-3108	7.43E-06	30.3	Activ at 10uM	
	SR-604559	NA	82.7	Activ at 10uM	
	SR-3111	2.65E-06	75		
	SR-3112	NA		Activ at 10uM	
	SR-3116	NA		Activ at 10uM	

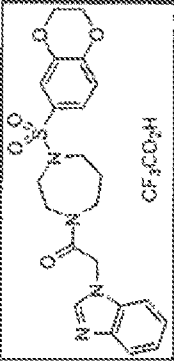
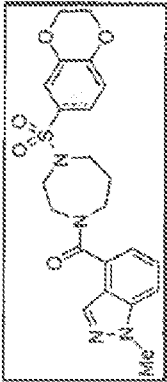
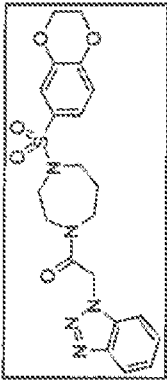
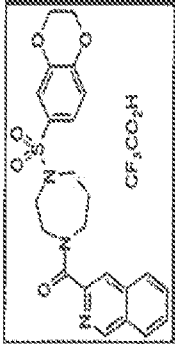
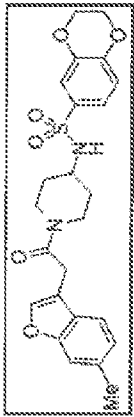
	SR-3117	5.01E-07	19.2	5.83E-06
	SR-3123	4.91E-06	58.7	1.63E-07
	SR-3124	NA		Activ at 10uM
	SR-3128	7.61E-06	82.3	Activ at 10uM
	SR-3129	NA		NA

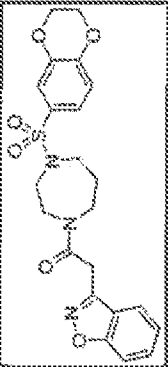
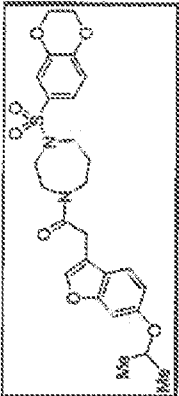
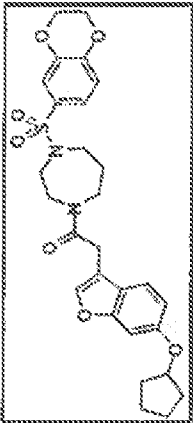
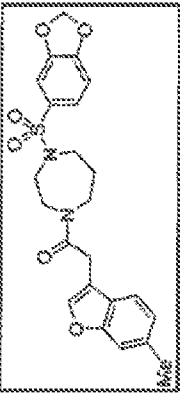
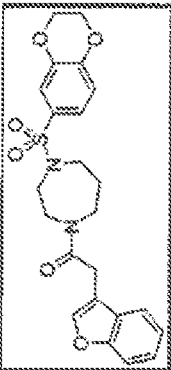
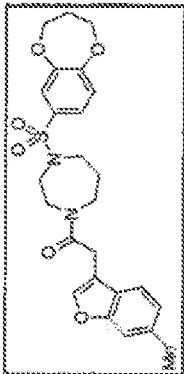
	SR-3133	NA	NA
	SR-3134	NA	NA
	SR-3139	NA	NA
	SR-604559	6.84E-05	
	SR-03000018417	NA	NA
	SR-03000018418	NA	NA

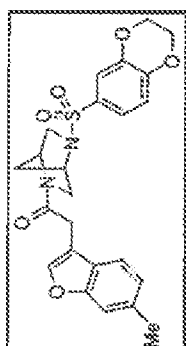
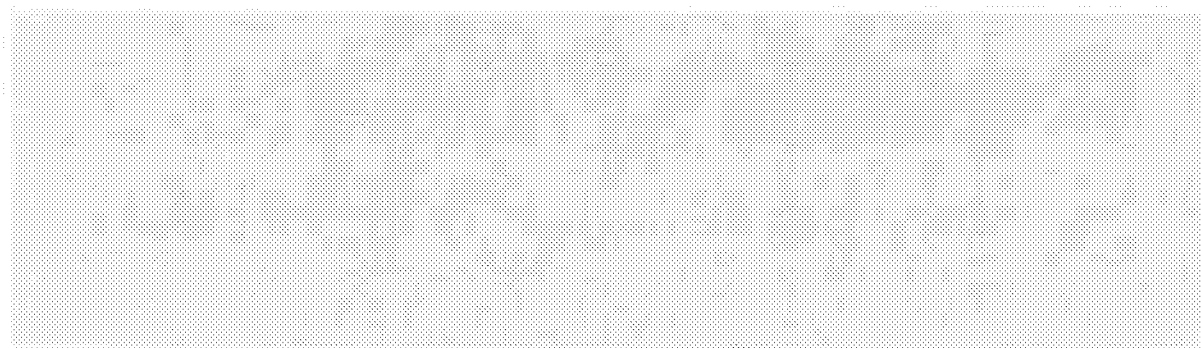
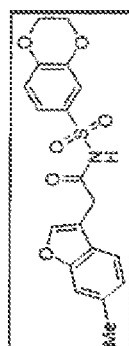
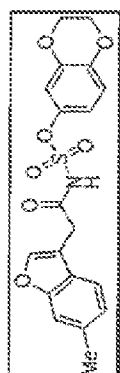
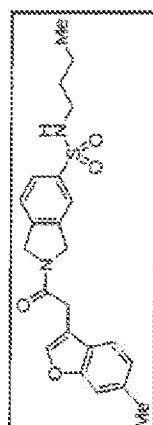
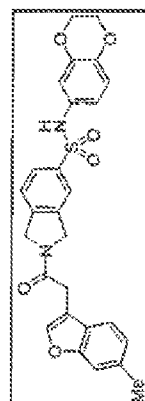
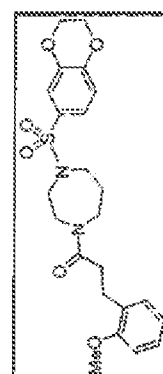
	SR-0000018419	NA	8.38E-06
	SR-0000018420	NA	1.56E-05
	SR-0000018421	NA	NA
	SR-0000018422	NA	4.05E-05
	SR-0000018423	NA	NA
	SR-0000018424	NA	4.01E-05

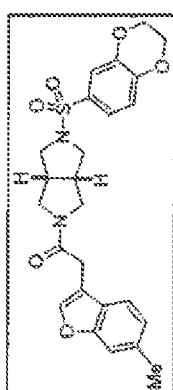
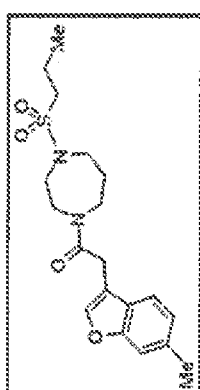
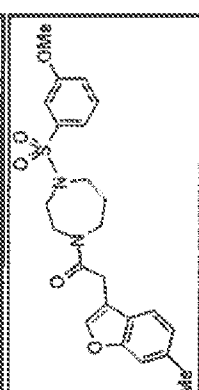
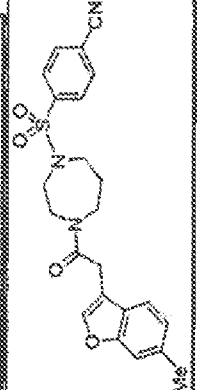
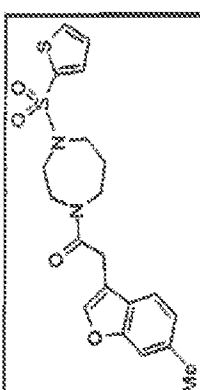
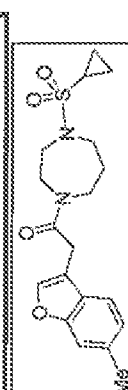
	SR-0000018425	1.84E-06	1.30E-07
	SR-0000018426	NA	3.31E-05
	SR-0000018427	NA	5.52E-06
	SR-0000018428	NA	NA
	SR-0000018429	NA	NA

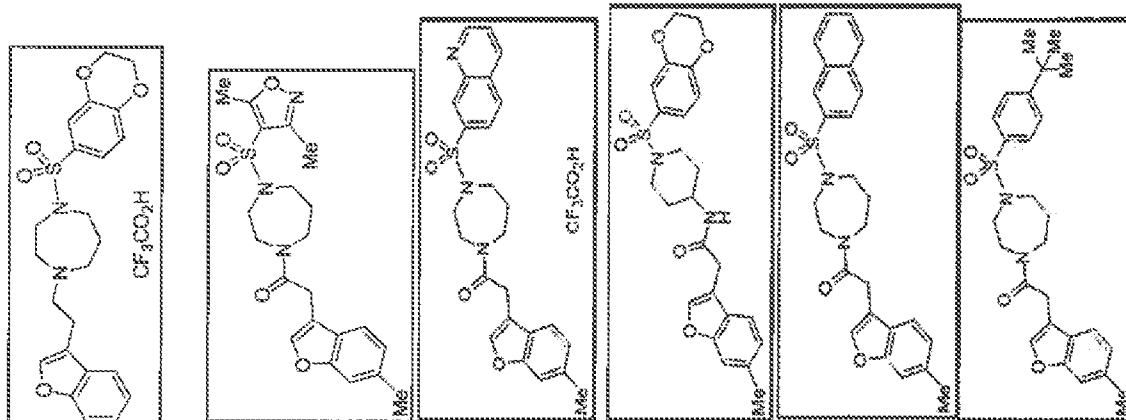
	SR-00000018430	NA	1.79E-05
	SR-00000018597	2.30E-06	8.69E-09
	SR-00000018598	NA	5.09E-06
	SR-00000018599	NA	NA
	SR-00000018600	NA	NA
	SR-00000018601	NA	NA

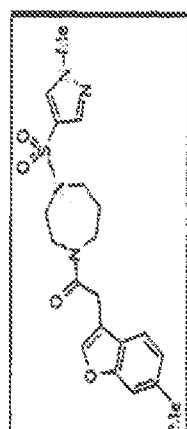
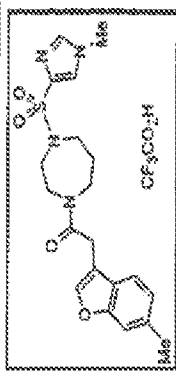
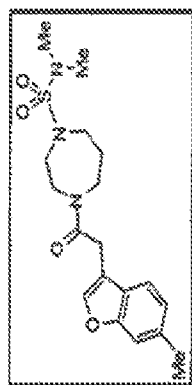
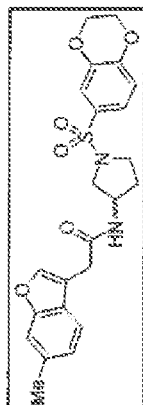
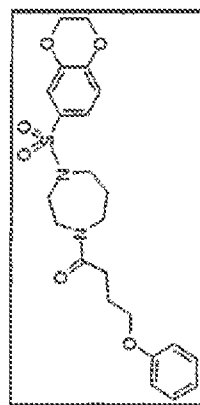
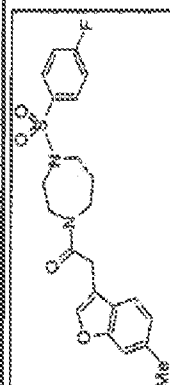
 SR-0000018602	NA	NA
 SR-0000018603	NA	NA
 SR-0000018604	NA	NA
 SR-0000018605	NA	NA
 SR-0000018606	5.38E-06	1.97E-07
 SR-0000018607	NA	2.71E-06

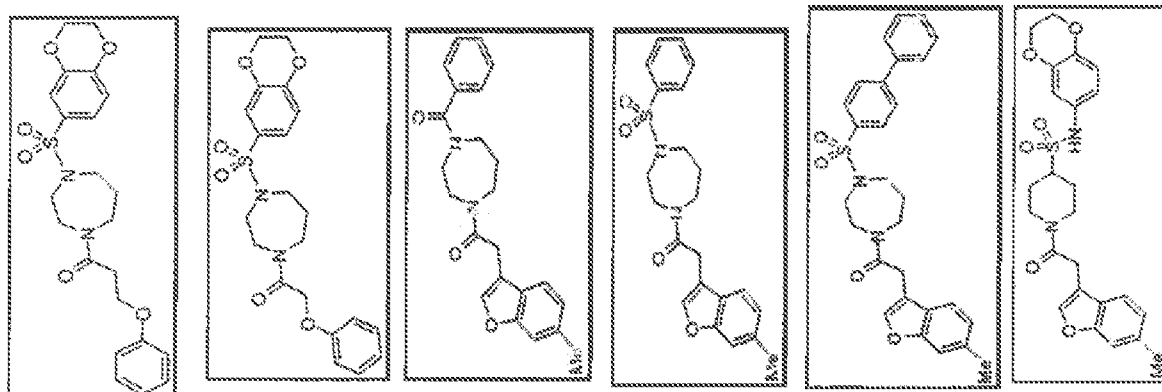
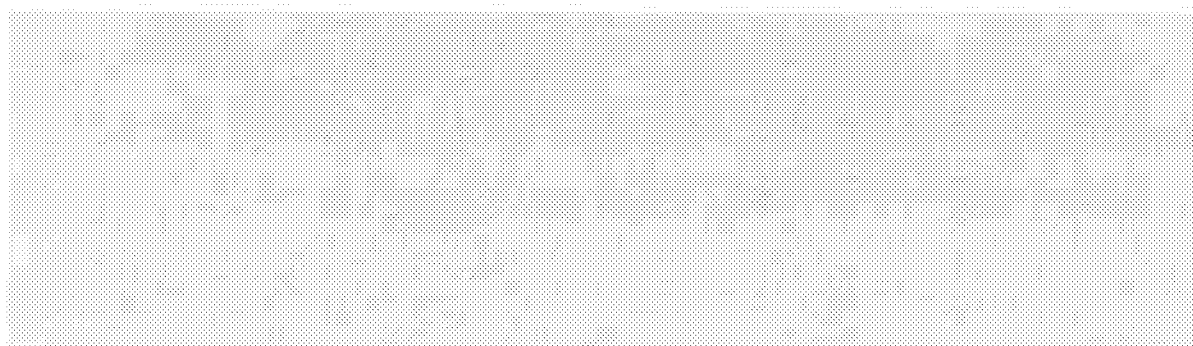
	SR- 00000018608	NA	2.07E-05
	SR- 00000018609	2.91E-06	4.89E-08
	SR- 00000018610	3.46E-06	1.93E-08
	SR- 00000018611	2.42E-06	1.23E-06
	SR- 00000018977		
	SR- 00000018978		

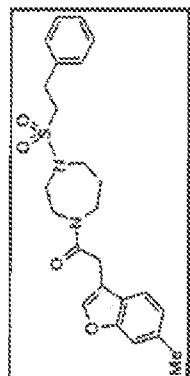
SR-
0000018979SR-
0000018980SR-
0000018981SR-
0000018982SR-
0000018983SR-
0000018984

SR-
0000018985SR-
0000018986SR-
0000018987SR-
0000018988SR-
0000018989SR-
0000018990

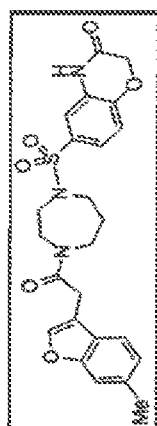


SR-
0000018997SR-
0000018998SR-
0000018999
NASR-
0000019000SR-
0000019001SR-
0000019002
NA

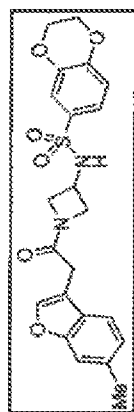


SR-
00000019241

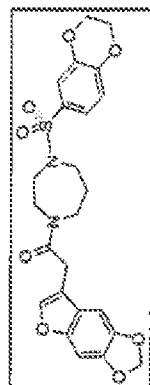
NA

SR-
00000019242

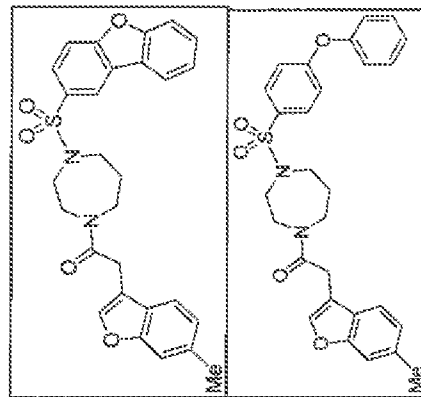
NA

SR-
00000019243

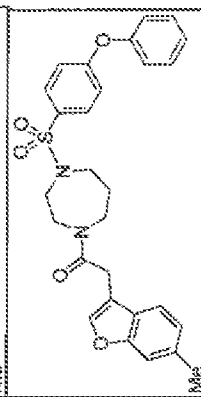
NA

SR-
00000019244

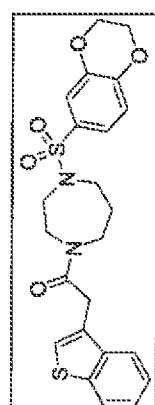
NA

SR-
00000019245

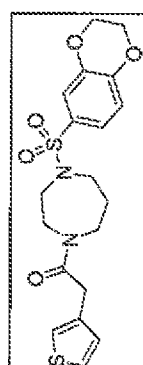
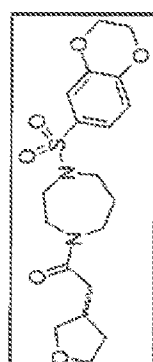
NA

SR-
00000019246

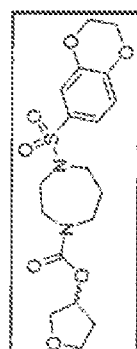
NA

SR-
00000019436

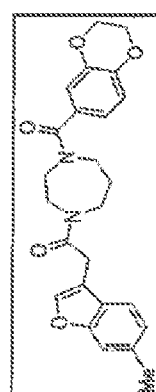
NA

inactive; 7%
activity IPO at 10
uMSR-
00000019437SR-
00000019438

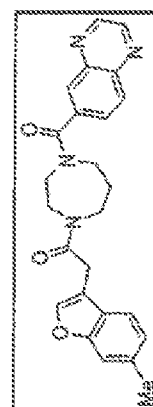
NA

SR-
00000019439

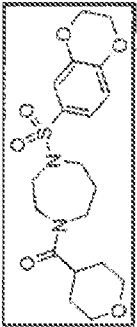
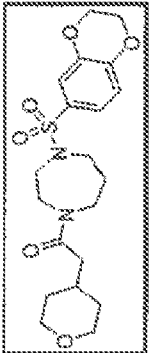
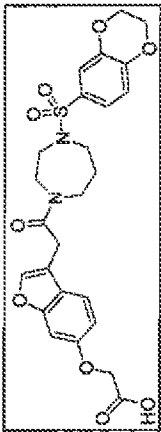
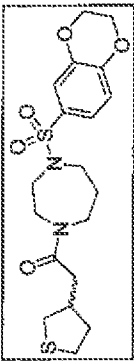
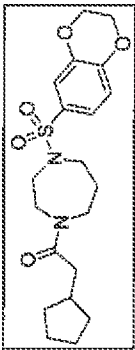
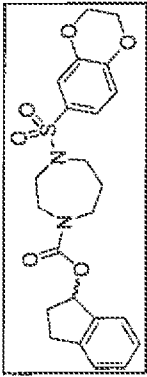
NA

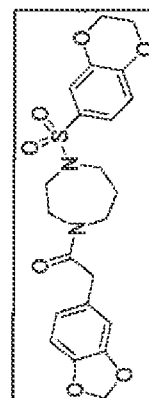
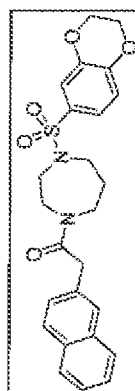
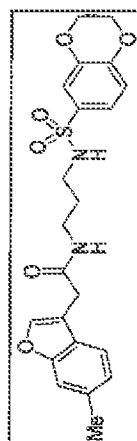
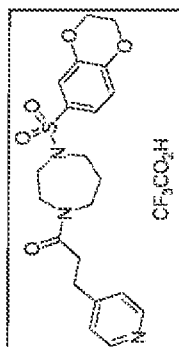
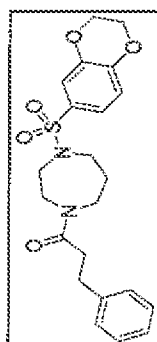
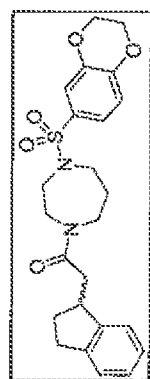
SR-
00000019440

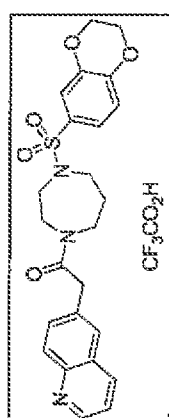
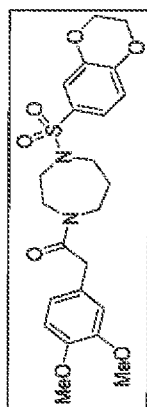
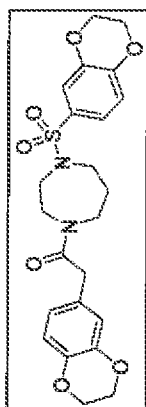
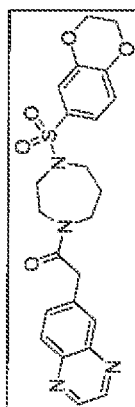
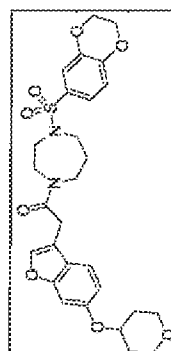
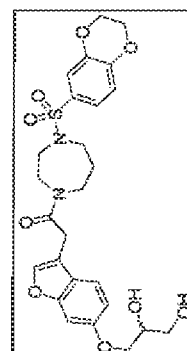
NA

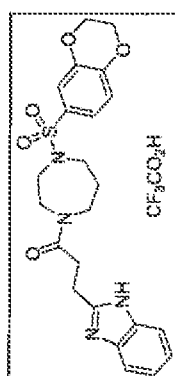
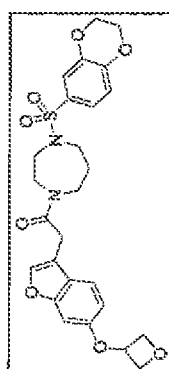
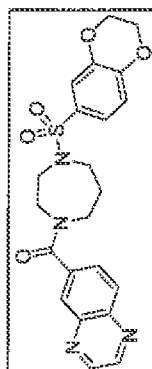
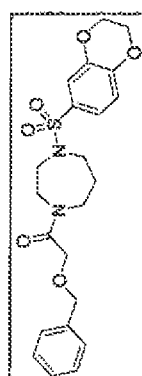
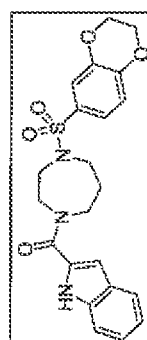
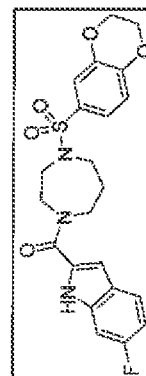
SR-
00000019441

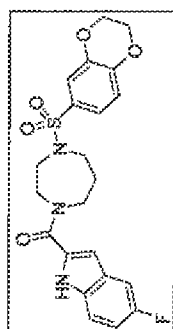
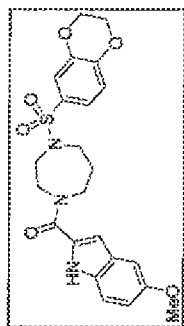
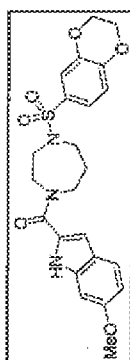
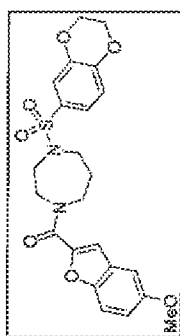
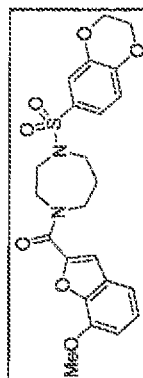
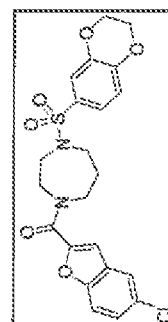
NA

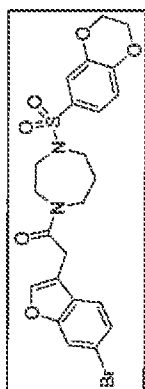
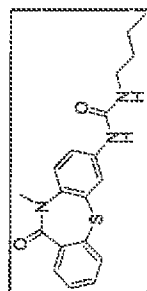
	SR-0000019442	NA
	SR-0000019443	NA
	SR-0000019444	NA
	SR-0000019445	NA
	SR-0000019446	inactive; 3% activity IPO at 10 uM
	SR-0000019738	



SR-
0000019745SR-
0000019746SR-
0000019747SR-
0000019748SR-
0000019918
active; > 1 μM SR-
0000019919

SR-
00000019920SR-
00000019737
active; > 1 μM SR-
00000019921SR-
00000019922SR-
00000019923SR-
00000019924

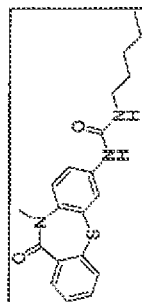
SR-
0000019925SR-
0000019926SR-
0000019927SR-
0000019928SR-
0000019929SR-
0000019930

SR-
0000019931active; > 1 μ M

SR-3178

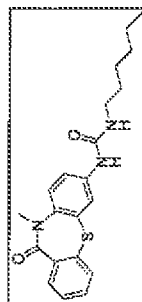
NA

NA



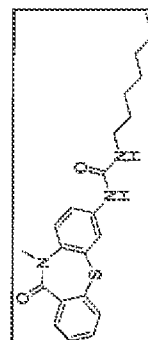
SR-3105

NA

Activ at 10 μ M

SR-3106

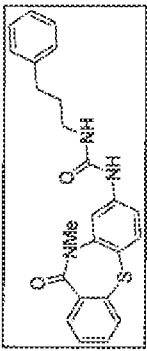
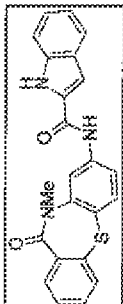
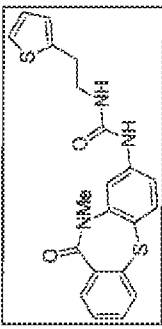
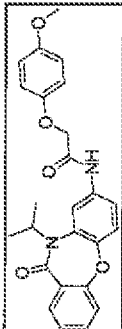
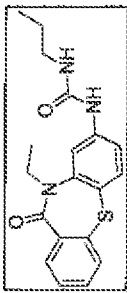
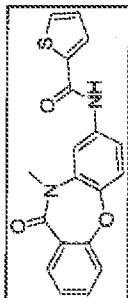
NA

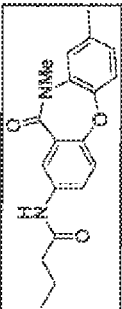
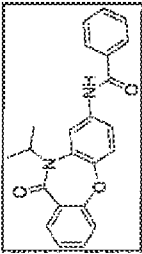
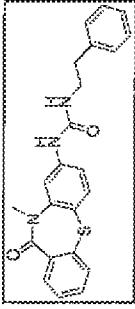
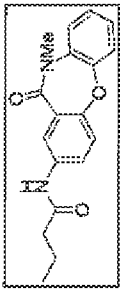
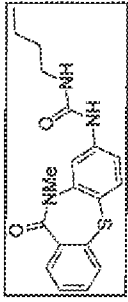
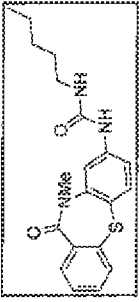
Activ at 10 μ M

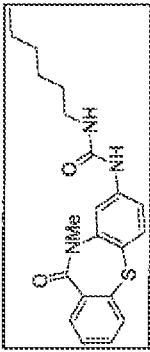
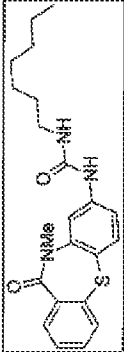
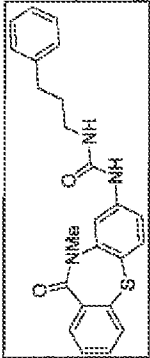
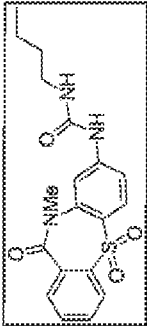
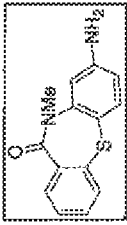
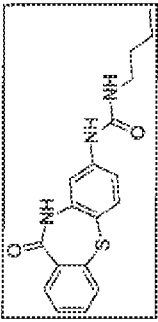
SR-3107

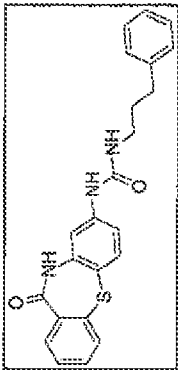
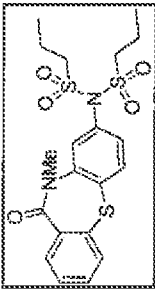
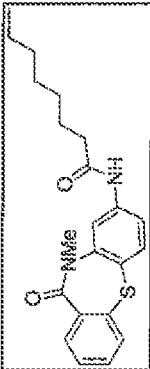
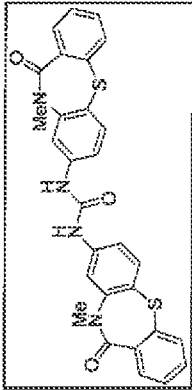
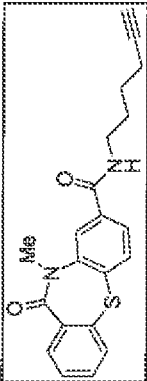
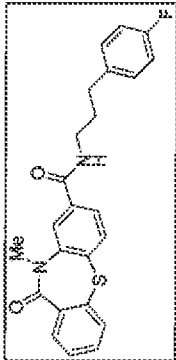
NA

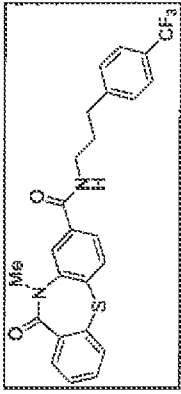
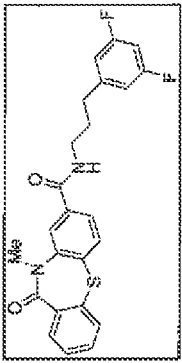
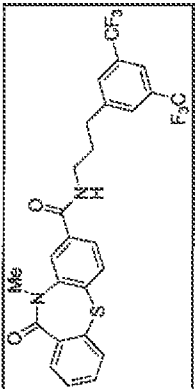
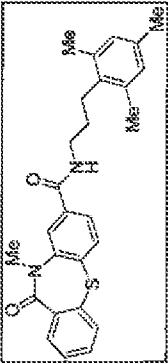
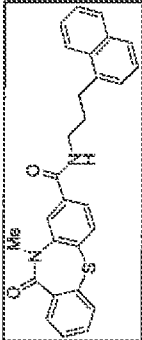
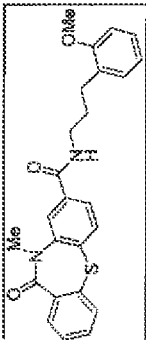
Activ at 10 μ M

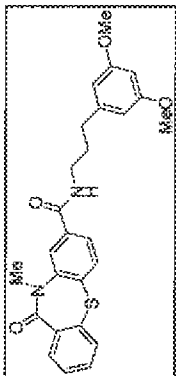
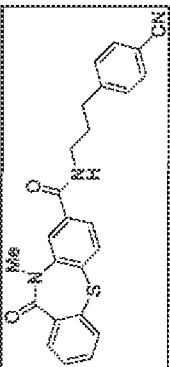
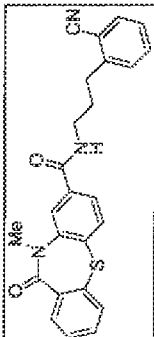
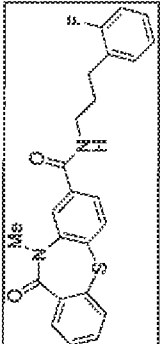
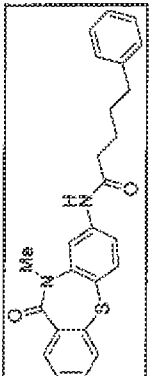
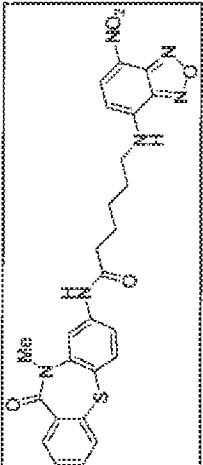
	SR-3114	1.58E-06	57.3	6.63E-09
	SR-3118	NA		NA
	SR-3119	NA		Activ at 10uM
	SR-3125	NA		NA
	SR-155338	1.72E-05	98	Activ at 10uM
	SR-3135	NA		NA

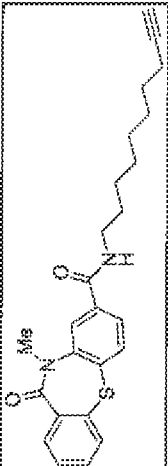
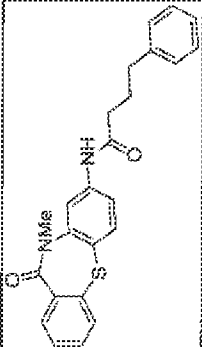
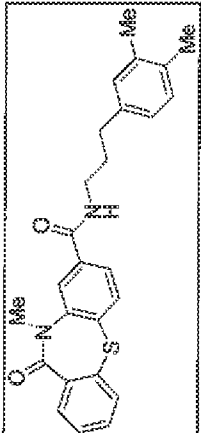
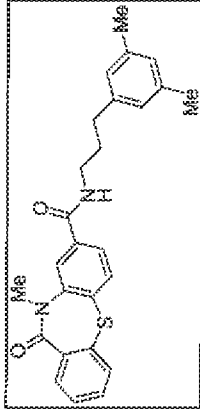
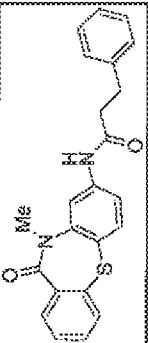
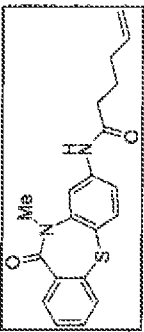
	SR-3140	NA	NA
	SR-3141	NA	NA
	SR-3146	NA	Actv at 10uM
	SR-3148	NA	NA
	SR-804995	8.57E-05	57, 74
	SR-3179	5.19E-05	49

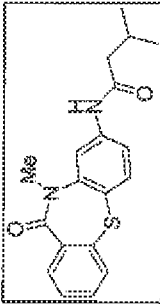
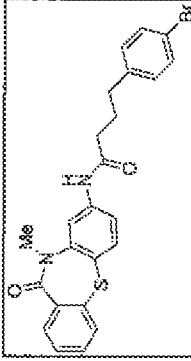
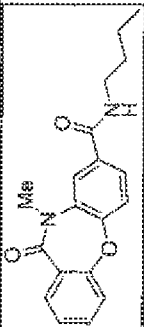
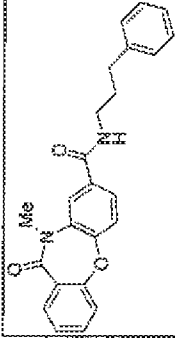
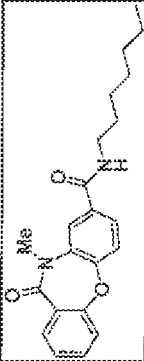
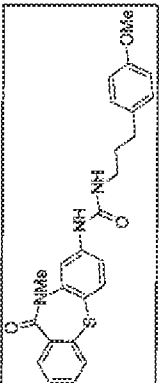
	SR-3180	5.16E-06	35
	SR-3182	4.80E-06	49
	SR-3114	1.10E-05	41 (57)
	SR-3265	NA	
	SR-3266	NA	
	SR-3267	NA	

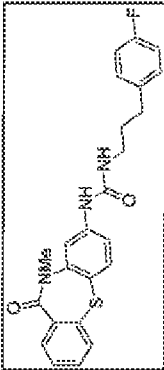
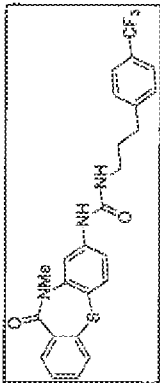
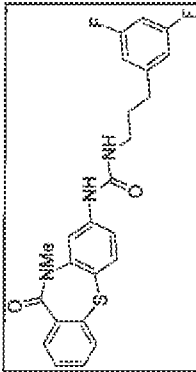
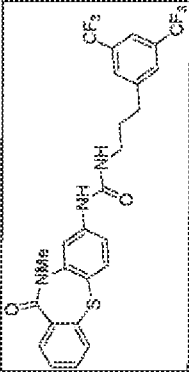
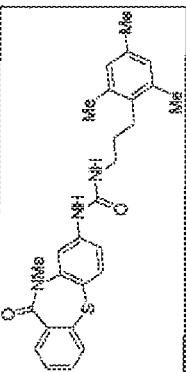
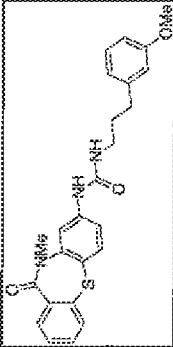
	SR-3268	NA				
	SR-3271	<15%				
	SR-3269	5.54E-07	40	3.83E-08	2.16E-08	
	SR-3270	NA				
	SR-3277	1.33E-05	61.5	4.03E-06	1.28E-06	
	SR-3279	3.97E-06	46	9.62E-07	6.25E-07	

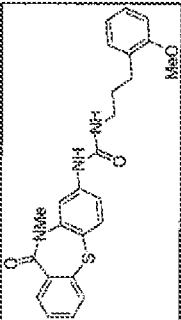
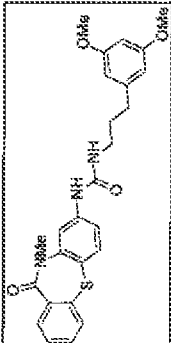
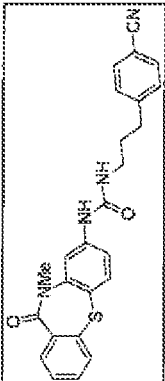
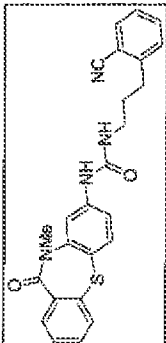
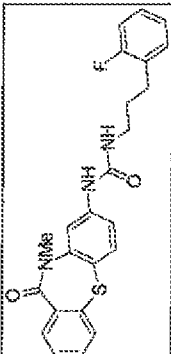
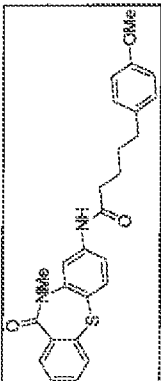
	SR-3280	2.81E-06	48	1.05E-06	3.72E-07
	SR-3281	6.29E-06	53	1.53E-06	4.68E-07
	SR-3282	3.42E-05	98	8.07E-07	3.40E-07
	SR-3283	3.98E-05	77.5	1.27E-06	4.90E-07
	SR-3284	1.49E-05	64	1.45E-06	5.82E-07
	SR-3287	1.67E-05	64	3.10E-06	1.05E-06

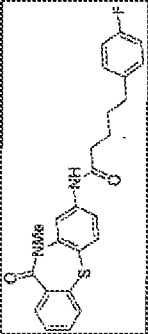
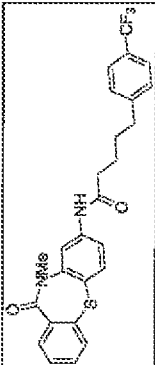
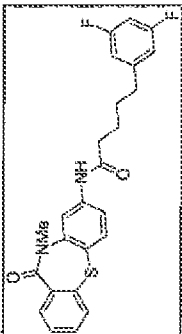
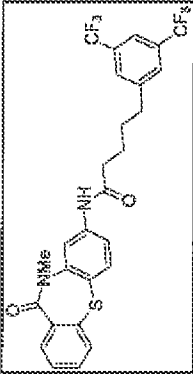
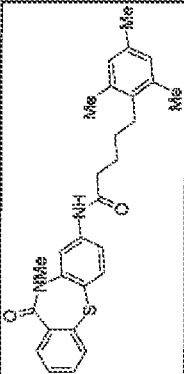
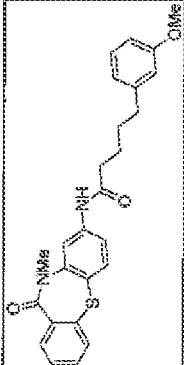
	SR-3288	2.64E-05	73.5	3.19E-06	1.04E-06
	SR-3289	3.46E-06	23.5	1.05E-06	1.37E-06
	SR-3290	1.99E-05	41	2.06E-06	1.81E-06
	SR-3291	3.37E-06	49.5	2.27E-06	7.60E-07
	SR-3294	1.21E-06	60	1.62E-07	5.19E-08
	SR-3295	1.89E-06	86.5	4.95E-07	2.16E-08

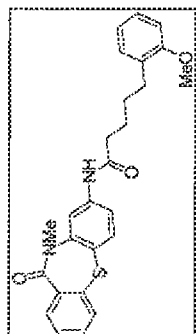
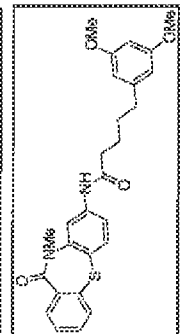
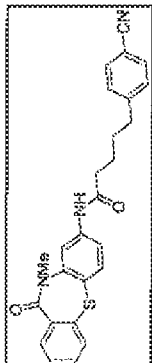
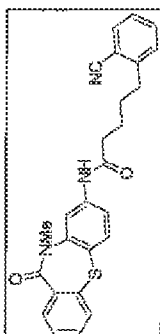
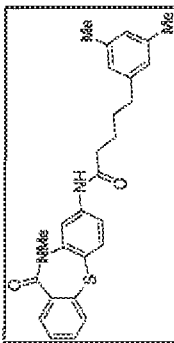
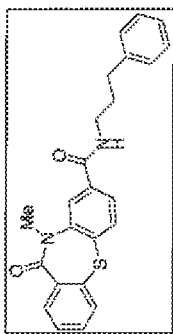
	SR-3292	4.98E-06	80	4.67E-07	2.95E-07
	SR-3293	NA		2.34E-07	9.06E-08
	SR-3321	1.98E-06	<20	1.07E-06	6.71E-07
	SR-3322	5.07E-06	80	1.60E-06	8.66E-07
	SR-3370	5.56E-06	40-60	7.33E-06	4.36E-07
	SR-3371	8.94E-06	40-60	8.85E-07	2.18E-07

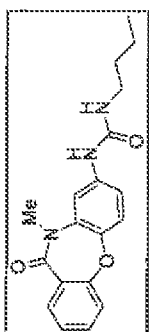
	SR-3372	4.61E-05	40-60	1.72E-06	5.43E-07
	SR-3373	1.18E-06	56	3.41E-07	1.09E-07
	SR-3374	NA		6.45E-05	
	SR-3375	NA		3.96E-06	
	SR-3376	NA		9.97E-07	
	SR-3416	3.20E-06	29	9.33E-08	

	SR-3417	5.48E-06	29	9.56E-07
	SR-3418	2.26E-06	42	1.41E-07
	SR-3419	5.45E-07	47	3.04E-08
	SR-3420	6.80E-07	67	2.15E-08
	SR-3421	1.28E-06	44	5.38E-08
	SR-3422	1.64E-06	46	8.15E-08

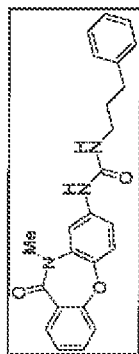
	SR-3423	4.04E-06	48	4.75E-08
	SR-3424	5.60E-06	45	1.46E-07
	SR-3425	NA		6.46E-08
	SR-3426	NA		1.97E-07
	SR-3427	4.36E-06	33	1.05E-07
	SR-3428	1.46E-06		1.46E-07

	SR-3429	4.02E-07	31	3.62E-08
	SR-3430	8.15E-07	61	3.62E-08
	SR-3431	4.38E-07		7.37E-08
	SR-3432	1.12E-06		6.11E-08
	SR-3433	3.77E-06		1.11E-07
	SR-3434	1.96E-06		1.87E-07

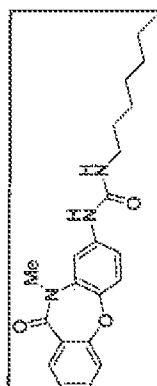
	SR-3435	1.93E-06	1.50E-07
	SR-3436	2.82E-06	2.13E-07
	SR-3437	3.26E-06	2.60E-07
	SR-3438	4.27E-06	2.52E-07
	SR-3440	1.46E-06	1.92E-07
	SR-3441	2.08E-06	1.29E-06



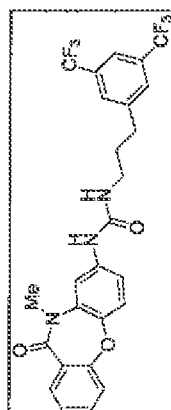
SR-3453



SR-3454



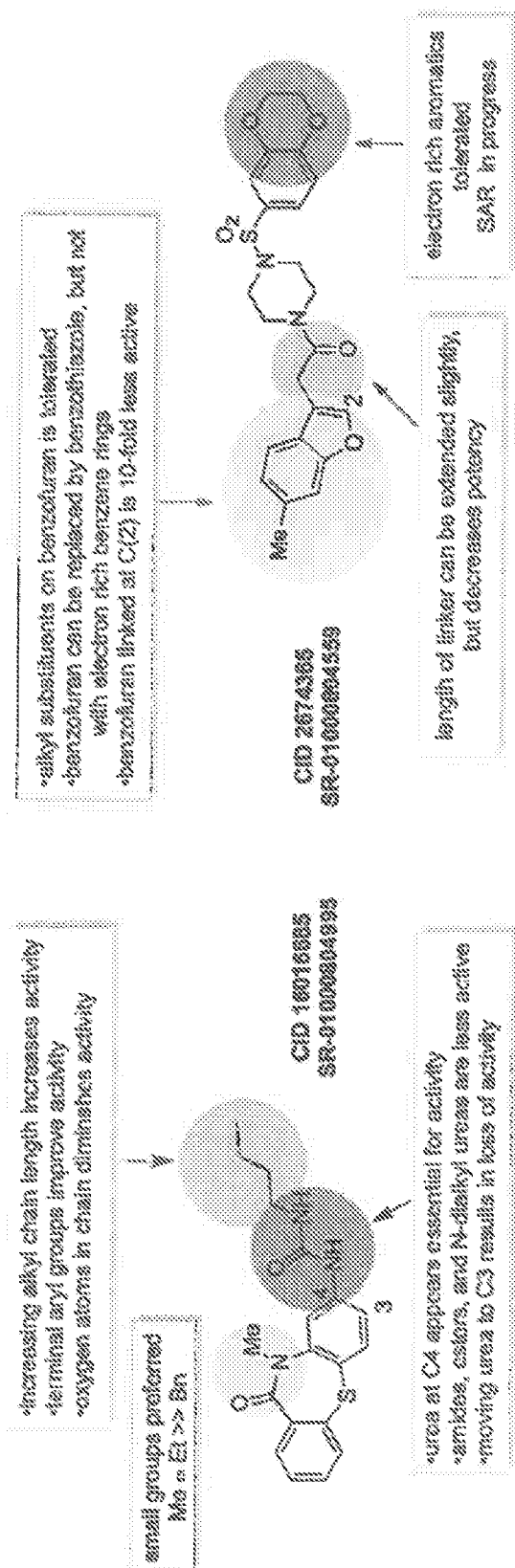
SR-3455



SR-3456

Scheme 1 shows some elements of the molecular design strategy based on the structural leads found by screening.

Scheme 1



All single enantiomer, diastereomeric, and racemic forms of a structure are intended, unless a particular stereochemistry or isomeric form is specifically indicated. In several instances though an individual stereoisomer is described among specifically claimed compounds, the stereochemical designation does not imply that alternate isomeric forms are less preferred, undesired, or not claimed. Compounds used in the present invention can include enriched or resolved optical isomers at any or all asymmetric atoms as are apparent from the depictions, at any degree of enrichment. Both racemic and diastereomeric mixtures, as well as the individual optical isomers can be isolated or synthesized so as to be substantially free of their enantiomeric or diastereomeric partners, and these are all within the scope of the invention.

Aryl groups are cyclic aromatic hydrocarbons that do not contain heteroatoms in the ring. An aromatic compound, as is well-known in the art, is a multiply-unsaturated cyclic system that contains $4n+2$ π electrons where n is an integer. Thus aryl groups include, but are not limited to, phenyl, azulenyl, heptalenyl, biphenyl, indacenyl, fluorenyl, phenanthrenyl, triphenylenyl, pyrenyl, naphthacenyl, chrysenyl, biphenylenyl, anthracenyl, and naphthyl groups. In some embodiments, aryl groups contain about 6 to about 14 carbons in the ring portions of the groups. Aryl groups can be unsubstituted or substituted.

Heteroaryl groups are aromatic ring compounds containing 5 or more ring members, of which, one or more is a heteroatom such as, but not limited to, N, O, and S; for instance, heteroaryl rings can be monocyclic ring systems having 5 or 6 ring members, or can be bicyclic ring systems, having 5:5, 5:6, or 6:6 configurations. In a bicyclic heteroaryl ring system, either one ring or both rings can contain one or more heteroatoms.

Standard abbreviations for chemical groups such as are well known in the art are used; e.g., Me = methyl, Et = ethyl, i-Pr = isopropyl, Bu = butyl, t-Bu = tert-butyl, Ph = phenyl, Bn = benzyl, Ac = acetyl, Bz = benzoyl, and the like.

The term "pharmaceutically acceptable salt" refers to salts which possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for

example utility in process of synthesis, purification or formulation of compounds of the invention. "Pharmaceutically or pharmacologically acceptable" include molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate. For human administration, preparations should meet sterility, pyrogenicity, and general safety and purity standards as required by FDA Office of Biologics standards.

If a value of a variable that is necessarily an integer, e.g., the number of carbon atoms in an alkyl group or the number of substituents on a ring, is described as a range, e.g., 0-4, what is meant is that the value can be any integer between 0 and 4 inclusive, i.e., 0, 1, 2, 3, or 4.

In various embodiments, the compound or set of compounds, such as are used in the inventive methods, can be any one of any of the combinations and/or sub-combinations of the above-listed embodiments.

In various embodiments, a compound as shown in any of the Examples, or among the exemplary compounds, is provided. Provisos may apply to any of the disclosed categories or embodiments wherein any one or more of the other above disclosed embodiments or species may be excluded from such categories or embodiments.

The compounds described herein can be prepared in a number of ways based on the teachings contained herein and synthetic procedures known in the art. In the description of the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the experiment and workup procedures, can be chosen to be the conditions standard for that reaction, unless otherwise indicated. It is understood by one skilled in the art of organic synthesis that the functionality present on various portions of the molecule should be compatible with the reagents and reactions proposed. Substituents not compatible with the reaction conditions will be apparent to one skilled in the art, and alternate methods are therefore indicated. The starting materials for the examples are either commercially available or are readily prepared by standard methods from known materials. All commercially available chemicals were obtained from Aldrich, Alfa Aesare, Wako, Acros, Fisher, Fluka, Maybridge or the like

and were used without further purification, except where noted. Dry solvents are obtained, for example, by passing these through activated alumina columns.

The present invention further embraces isolated compounds of the invention. The expression "isolated compound" refers to a preparation of a compound of the invention, or a mixture of compounds of the invention, wherein the isolated compound has been separated from the reagents used, and/or byproducts formed, in the synthesis of the compound or compounds. "Isolated" does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to compound in a form in which it can be used therapeutically. Preferably an "isolated compound" refers to a preparation of a compound of the invention or a mixture of compounds of the invention, which contains the named compound or mixture of compounds of the invention in an amount of at least 10 percent by weight of the total weight. Preferably the preparation contains the named compound or mixture of compounds in an amount of at least 50 percent by weight of the total weight; more preferably at least 80 percent by weight of the total weight; and most preferably at least 90 percent, at least 95 percent or at least 98 percent by weight of the total weight of the preparation.

The compounds of the invention and intermediates may be isolated from their reaction mixtures and purified by standard techniques such as filtration, liquid-liquid extraction, solid phase extraction, distillation, recrystallization or chromatography, including flash column chromatography, or HPLC.

Another aspect of an embodiment of the invention provides compositions of the compounds of the invention, alone or in combination with another medicament. As set forth herein, compounds of the invention include stereoisomers, tautomers, solvates, prodrugs, pharmaceutically acceptable salts and mixtures thereof. Compositions containing a compound of the invention can be prepared by conventional techniques, e.g. as described in Remington: *The Science and Practice of Pharmacy*, 19th Ed., 1995, or later versions thereof, incorporated by reference herein. The compositions can appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

"Treating" or "treatment" within the meaning herein refers to an alleviation of symptoms associated with a disorder or disease, or inhibition of further progression or

worsening of those symptoms, or prevention or prophylaxis of the disease or disorder, or curing the disease or disorder. Similarly, as used herein, an "effective amount" or a "therapeutically effective amount" of a compound of the invention refers to an amount of the compound that alleviates, in whole or in part, symptoms associated with the disorder or condition, or halts or slows further progression or worsening of those symptoms, or prevents, or provides prophylaxis for, the disorder or condition. In particular, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount is also one in which any toxic or detrimental effects of compounds of the invention are outweighed by the therapeutically beneficial effects.

It is within ordinary skill to evaluate any compound disclosed and claimed herein for effectiveness using the procedures described above or found in the scientific literature. Accordingly, the person of ordinary skill can prepare and evaluate any of the claimed compounds without undue experimentation.

Any compound found to be an effective agent for disrupting a PLIN1-ABHD5 complex to stimulate adipocyte lipolysis can likewise be tested in animal models and in human clinical studies using the skill and experience of the investigator to guide the selection of dosages and treatment regimens.

A typical dosage is about 10 mg to about 1000 mg per day. In choosing a regimen for patients it can frequently be necessary to begin with a higher dosage and when the condition is under control to reduce the dosage. The exact dosage will depend upon the activity of the compound, mode of administration, on the therapy desired, form in which administered, the subject to be treated and the body weight of the subject to be treated, and the preference and experience of the physician in charge.

Examples

High throughput compound screening: Luciferase complementation constructs were generated as previously described^{7,14,29} and proteins were produced in 293T or Sf9 cells. High throughput compound screening was performed at by the Molecular Screening Center at the Scripps Research Institute in Jupiter Florida. The assays and primary screening methods and results are detailed in PubChem AID 453907, AID 651674.

Molecular and biochemical techniques for secondary analyses: Cellular lysates were prepared from transfected 293T cells^{7,14} and complementation assays were performed in 96 well plates using 50 µl of each lysate and 1 µl of compounds dissolved in DMSO or DMSO vehicle. Luciferase activity was determined after incubation for 4 hours at room temperature. Immunoblotting was performed as previously described.¹⁶ In vitro lipolysis assays were performed using ABHD5 purified from Sf9 cells and ATGL overexpressed in Cos7 cells, as detailed.³³ Cloning and mutagenesis were performed using PCR, and all constructs were confirmed by sequencing.

Analysis of brown adipocyte lipolysis: Immortalized brown adipocytes were seeded in 96 well plates and differentiated, as described.¹⁶ Three to four days after induction of differentiation, cells were washed and placed in HEPES-buffered Krebs Ringer buffer containing 1% bovine serum albumin, and treated with DMSO (vehicle) or activators at concentrations specified in the figures. Accumulated fatty acids or glycerol were determined using WAKO kits, as described.¹⁶

Stable knockdown and inducible rescue of ABHD5 in brown adipocytes: Lentiviral shRNA to Abhd5 was created from a plasmid purchased from Sigma (TRCN0000032737 NM_026179.1-1063s1c1), and used to stably transduce mouse brown adipocytes. Knockdown was confirmed by immunoblotting of differentiated adipocytes. To create an inducible rescue cell line we performed silent mutagenesis of three amino acids targeted by the shRNA and tagged the construct with mCherry. Doxycycline-inducible expression was achieved by transferring the shRNA-resistant construct into pINDUCER20,³⁴ and resulting lentivirus used to infect the brown adipocytes in which endogenous ABHD5 was stably knocked down.

Imaging compound action in live cells: Full-length human ABHD5 and a fragment of human PLIN1 encoding amino acids 358 to 417 were amplified by PCR and cloned into the expression vectors ECFP-C1 and EYFP-N1, respectively. Cos7 cells were plated onto coverslips, and transfected and loaded with oleic acid, as previously described.^{7,29} For imaging of fatty acid production in live cells, we created a brown adipocyte cell line that expresses a fatty acid reporter system¹⁹ under the control of doxycycline-sensitive promoter. In this system, PLIN1 is fused to ligand binding domain (LBD) of PPARα, thus targeting the fatty acid-sensing domain of PPARα to lipid droplets. Mobilized fatty acids that are bound by the PPARα LBD are detected by the accumulation of EYFP-tagged SRC1 coactivator binding domain at the lipid droplet surface.

Covalent labeling of ABHD5 with NBD-HE-HP (NDB) and mass-spectrometry of labeled peptides: NBD affinity label was synthesized and purified as described.²⁰ Cos7 cells transfected with PLIN1 and wild type or mutant ABHD5 were lipid loaded overnight with 200 µM oleic acid.

One day after transfection, cells labeled with 50 μ M NBD-HE-HP in serum-free DMEM for one hour at 37°C. Cells were rinsed with PBS and protein-matched aliquots of cell lysates were separated by SDS-PAGE. Two gels were run in parallel, one for fluorescent scanning and one for immunoblot detection of ABHD5. Gels for scanning were fixed (45% MeOH, 10% acetic acid) then visualized on a Typhoon 9410 Variable Mode Imager (GE Healthcare; excitation 488 nm, emission 520 nm).

LC/MS/MS characterization of NBD-HE-HP labeled ABHD5: 293-T cells transfected with His-tagged ABHD5 were labeled with NBD in serum-free DMEM for 3 hours at 37°C. His-tagged ABHD5 was partially purified by His60 Ni²⁺ Superflow Resin column chromatography (Clontech), as described by the manufacturer. Labeled ABHD5 was further resolved by SDS-PAGE and gel bands containing labeled ABHD5 were subjected to LC/MS analysis. Proteins were reduced, alkylated, and digested with either trypsin or chymotrypsin in-gel, and separated by reverse phase chromatography. In the first experiment, peptides were analyzed with a LTQ-XL linear ion trap mass spectrometer (Thermo Fisher). Settings included: dynamic exclusion turned on (1 ion in 5s then excluded for 10s, 200 list size); top 7 ions fragmented by collision-induced dissociation (CID) if above a 500 threshold; Normalized Collision Energy = 35%; and Activation Q = 0.25. In the second experiment, samples were analyzed in an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher). All scans were performed at a resolution of 120,000. Ions with charge states of 2 or 3 were fragmented by CID (35% collision energy), and those with higher charge states were fragmented with electron transfer dissociation (ETD). Dynamic exclusion was turned on (1 ion in 30 s, then excluded for 60s). Peak lists were generated with Proteome Discoverer 1.4 (Thermo) and peptide assignments were scored against a mouse protein database (Swissprot; March 2014; 16722 entries) using Mascot (Matrix Sciences, ver 2.4). Static modification of +57 (carbamidomethylation, C) and dynamic modifications of -17 (acetylation, N-terminal), +18 (oxidation, M), +1 (deamidation, NQ) and +467 (NBD modification, ADEGHILSTY) were included. Mascot data was imported into Scaffold 4.3 (Proteome Software) which reanalyzed a subset protein list using XITandem (ver 2010.12.01.1), and calculated peptide and probabilities using Peptide Prophet. Peptides were scored as positive if they scored above a 99% threshold.

Documents Cited:

1. Arch, J.R., *et al.* Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* **309**, 163-165 (1984).
2. Cummings, D.E., *et al.* Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature* **382**, 622-626 (1996).

3. Robidoux, J., Martin, T.L. & Collins, S. Beta-adrenergic receptors and regulation of energy expenditure: a family affair. *Annual review of pharmacology and toxicology* **44**, 297-323 (2004).
4. Granneman, J.G. & Moore, H.P. Location, location: protein trafficking and lipolysis in adipocytes. *Trends Endocrinol Metab* **19**, 3-9 (2008).
5. Young, S.G. & Zechner, R. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. *Genes & development* **27**, 459-484 (2013).
6. Lass, A., et al. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chananin-Dorfman Syndrome. *Cell metabolism* **3**, 309-319 (2006).
7. Granneman, J.G., Moore, H.P., Krishnamoorthy, R. & Rathod, M. Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (Atgl). *J Biol Chem* **284**, 34538-34544 (2009).
8. Ahmadian, M., et al. Adipose overexpression of desnutrin promotes fatty acid use and attenuates diet-induced obesity. *Diabetes* **58**, 855-866 (2009).
9. Arch, J.R.S. β 3-Adrenoceptor agonists: potential, pitfalls and progress. *Eur J of Pharmacol* **440**, 99-107 (2002).
10. Caux, F., Selma, Z.B., Laroche, L., Prud'homme, J.F. & Fischer, J. CGI-58/ABHD5 gene is mutated in Dorfman-Chananin syndrome. *American journal of medical genetics. Part A* **129A**, 214 (2004).
11. Fischer, J., et al. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. *Nature genetics* **39**, 28-30 (2007).
12. Reilich, P., et al. The phenotypic spectrum of neutral lipid storage myopathy due to mutations in the PNPLA2 gene. *Journal of neurology* **258**, 1987-1997 (2011).
13. Schweiger, M., Lass, A., Zimmermann, R., Eichmann, T.O. & Zechner, R. Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5. *American journal of physiology. Endocrinology and metabolism* **297**, E289-296 (2009).
14. Granneman, J.G., Moore, H.P., Mottillo, E.P. & Zhu, Z. Functional interactions between Mldp (LSDP5) and Abhd5 in the control of intracellular lipid accumulation. *J Biol Chem* **284**, 3049-3057 (2009).
15. Pollak, N.M., et al. Cardiac-specific overexpression of perilipin 5 provokes severe cardiac steatosis via the formation of a lipolytic barrier. *Journal of lipid research* **54**, 1092-1102 (2013).

16. Wolins, N.E., *et al.* OXPAT/PAT-1 Is a PPAR-Induced Lipid Droplet Protein That Promotes Fatty Acid Utilization. *Diabetes* **55**, 3418-3428 (2006).
17. Remy, I. & Michnick, S.W. A highly sensitive protein-protein interaction assay based on Gaussia luciferase. *Nature methods* **3**, 977-979 (2006).
18. Mottillo, E.P., Bloch, A.E., Leff, T. & Granneman, J.G. Lipolytic Products Activate Peroxisome Proliferator-activated Receptor (PPAR) α and δ in Brown Adipocytes to Match Fatty Acid Oxidation with Supply. *Journal of Biological Chemistry* **287**, 25038-25048 (2012).
19. Mottillo, E.P., Paul, G.M., Moore, H.P. & Granneman, J.G. Use of fluorescence microscopy to probe intracellular lipolysis. *Methods in enzymology* **538**, 263-278 (2014).
20. Birner-Gruenberger, R. & Hermetter, A. Activity-based proteomics of lipolytic enzymes. *Current drug discovery technologies* **4**, 1-11 (2007).
21. Yen, C.L. & Farese, R.V., Jr. Fat breakdown: a function for CGI-58 (ABHD5) provides a new piece of the puzzle. *Cell metabolism* **3**, 305-307 (2006).
22. Gu, C., *et al.* Chemical proteomics with sulfonyl fluoride probes reveals selective labeling of functional tyrosines in glutathione transferases. *Chemistry & biology* **20**, 541-548 (2013).
23. Heath, R.J. & Rock, C.O. A conserved histidine is essential for glycerolipid acyltransferase catalysis. *Journal of bacteriology* **180**, 1425-1430 (1998).
24. Lewin, T.M., Wang, P. & Coleman, R.A. Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. *Biochemistry* **38**, 5764-5771 (1999).
25. Zierler, K.A., Zechner, R. & Haemmerle, G. Comparative gene identification-58/alpha/beta hydrolase domain 5: more than just an adipose triglyceride lipase activator? *Current opinion in lipidology* **25**, 102-109 (2014).
26. McMahon, D., *et al.* Comparative gene identification 58/alpha/beta hydrolase domain 5 lacks lysophosphatidic acid acyltransferase activity. *Journal of lipid research* **55**, 1750-1761 (2014).
27. Miyoshi, H., *et al.* Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated lipolysis in adipocytes. *J Biol Chem* **282**, 996-1002 (2007).
28. Miyoshi, H., *et al.* Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. *J Biol Chem* **281**, 15837-15844 (2006).
29. Granneman, J.G., Moore, H.P., Mottillo, E.P., Zhu, Z. & Zhou, L. Interactions of perilipin-5 (Plin5) with adipose triglyceride lipase. *J Biol Chem* **286**, 5126-5135 (2011).

30. Fedorenko, A., Lishko, P.V. & Kirichok, Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* **151**, 400-413 (2012).
31. Guerra, C., *et al.* Abnormal nonshivering thermogenesis in mice with inherited defects of fatty acid oxidation. *The Journal of clinical investigation* **102**, 1724-1731 (1998).
32. Nicholls, D.G. The physiological regulation of uncoupling proteins. *Biochim Biophys Acta* **1757**, 459-466 (2006).
33. Schweiger, M., *et al.* Measurement of lipolysis. *Methods in enzymology* **538**, 171-193 (2014).
34. Meerbrey, K.L., *et al.* The pINDUCER lentiviral toolkit for inducible RNA interference in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 3665-3670 (2011).

While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements will be apparent to those skilled in the art without departing from the spirit and scope of the claims.

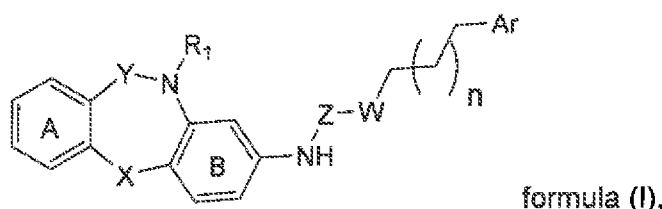
All patents and publications referred to herein are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A method of stimulating cellular lipolysis by disrupting complexes of PLIN1-ABHD5 or PLIN5-ABHD5, comprising contacting ABHD5 with an effective amount or concentration of a compound wherein the compound is of formula (I)



wherein

ring A and ring B are each independently aryl, 5-membered heteroaryl, or 6-membered heteroaryl;

wherein ring A and ring B each optionally bear 1-3 independently selected V, where each V is independently at each occurrence halogen, cyano, nitro, CF₃, lower alkyl, lower alkoxy, CO₂H, CO₂R, or CONR₂;

X and W are each independently S, O, NR or CR, wherein R is H, alkyl or aryl;

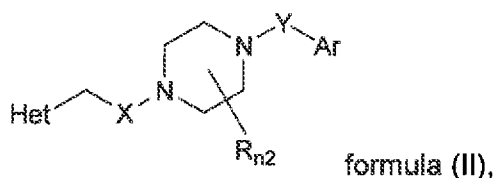
Y and Z are each independently CO or SO₂;

Ar is substituted aryl or heteroaryl;

n = 0-5;

or a pharmaceutically acceptable salt thereof;

or wherein the compound is of formula (II)



wherein

Het is a 5-membered heteroaryl, a 6-membered heteroaryl, or a bicyclic heteroaryl;

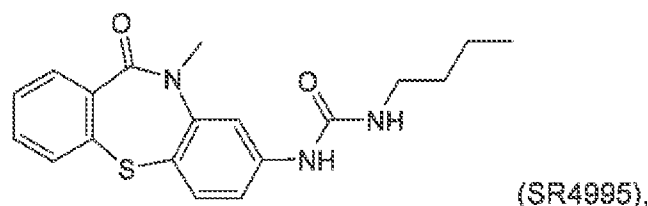
Ar is aryl or heteroaryl;

wherein Het and Ar each optionally bear 1-3 independently selected V, where each V is independently at each occurrence halogen, cyano, nitro, CF₃, lower alkyl, lower alkoxy, CO₂H, CO₂R, or CONR₂;

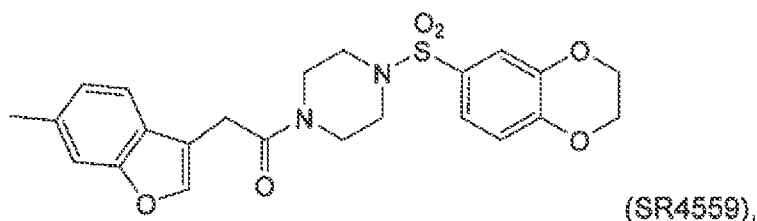
X and Y are each independently CO or SO₂;

or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the compound is



or



or a pharmaceutically acceptable salt thereof.

3. A method of promoting fat catabolism, comprising administering to a patient an effective dose of a compound of formula (I) or formula (II) of claim 1.
4. A method of treatment of obesity, diabetes, cardiovascular disease, or cancer, comprising administering to a patient afflicted therewith an effective dose of a compound of formula (I) or formula (II) of claim 1.
5. A method of treatment of ichthyosis, comprising applying to the skin of a patient afflicted therewith an effective amount of a topical formulation comprising a compound of formula (I) or formula (II) of claim 1.
6. A method of increasing the content in skin of skin barrier lipids, comprising applying to skin an effective amount of a topical formulation comprising a compound of formula (I) or formula (II) of claim 1.

FIGURES

Figure 1

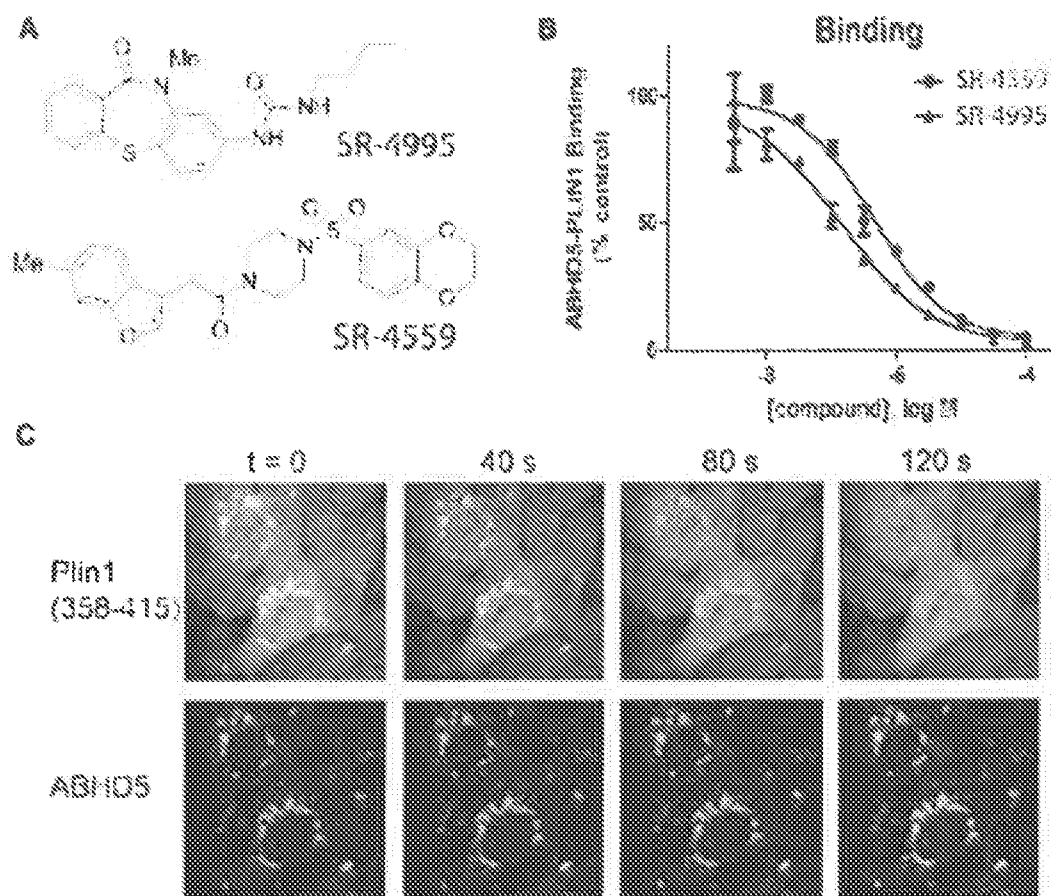


Figure 2

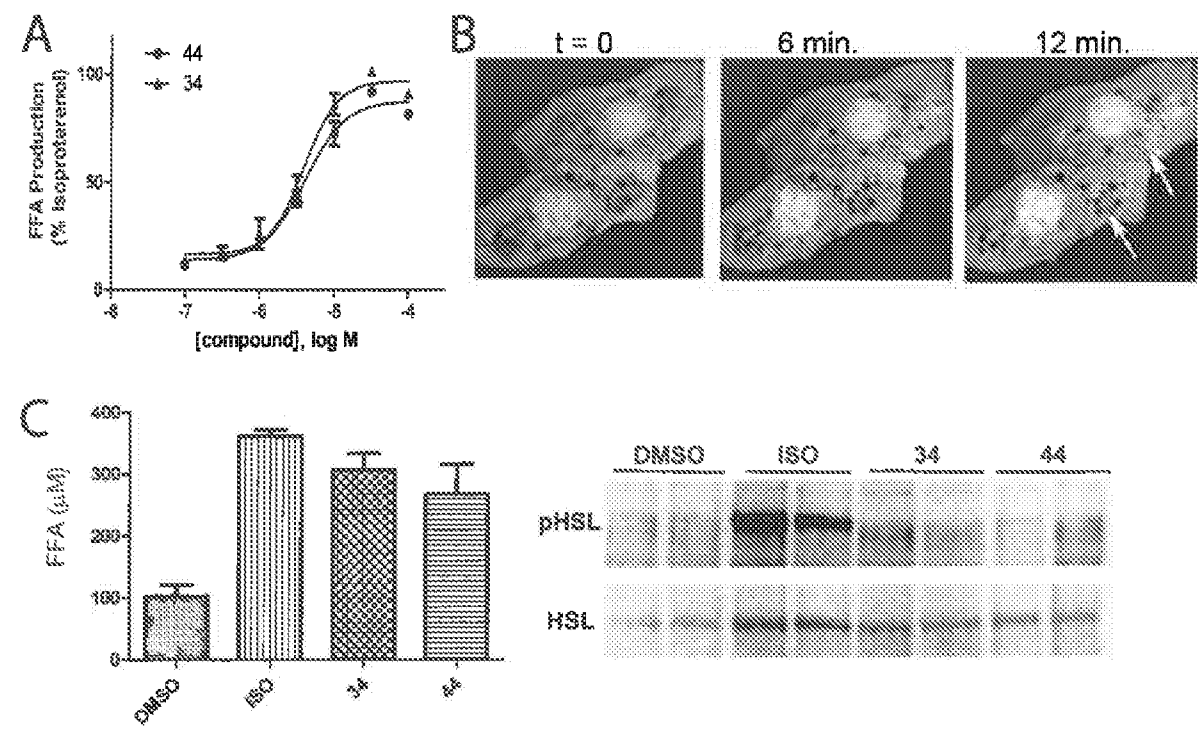


Figure 3

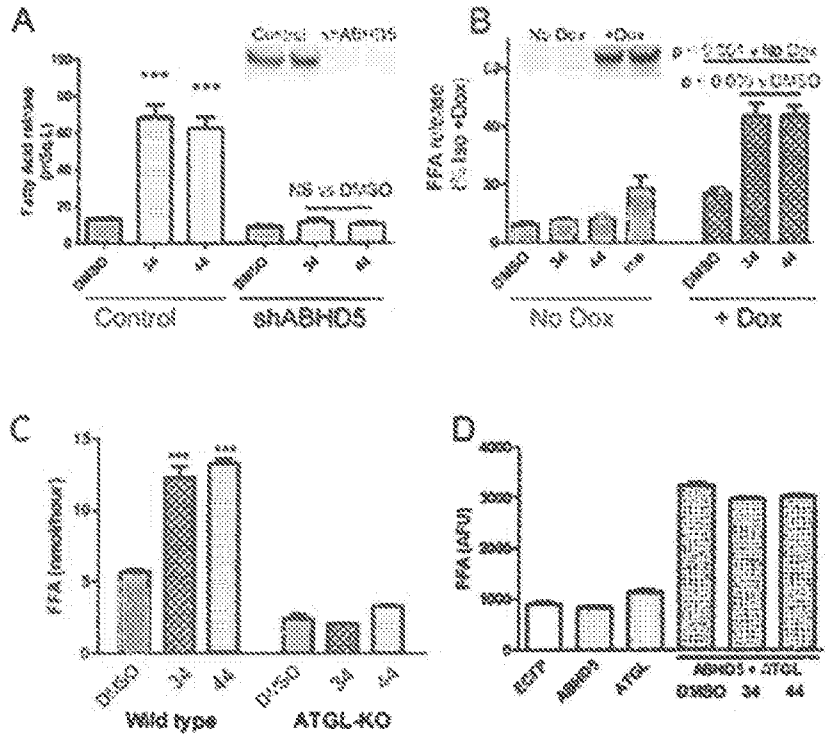
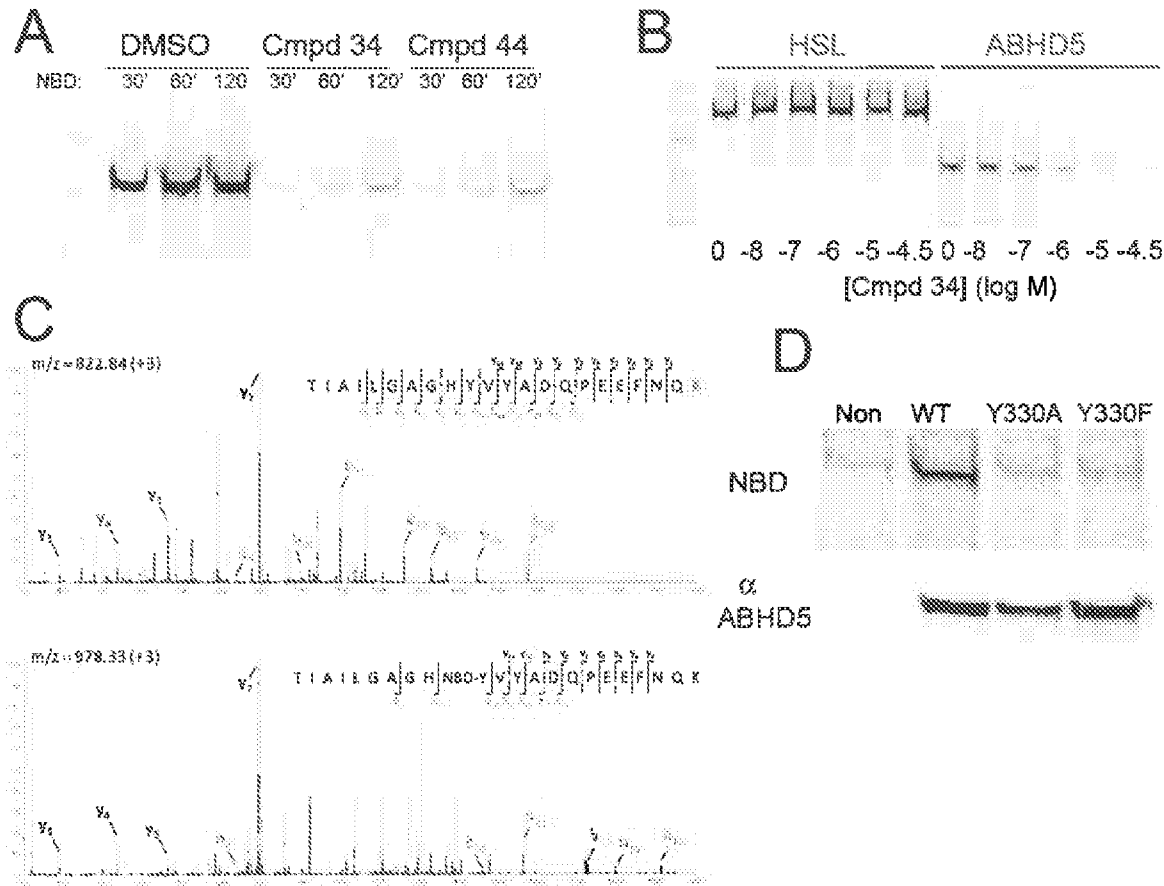


Figure 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/53005

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 36/82; A61P 3/04 (2016.01)

CPC - A61K 36/82; C07D 295/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 36/82; A61P 3/04 (2016.01)

CPC: A61K 36/82; C07D 295/26

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO; PubMed; Google Scholar; lipolysis, complex, PLIN1, PLIN5, ABHD5, skin, cancer, lipid, fat, catabolism, obesity, ichthyosis, topical, barrier, dry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2012/0165324 A1 (SALITURO, FG et al.) 28 June 2012; paragraphs [0007]-[0014], [0019], [0031]	4 ----- 1-3, 5-6
Y	PATEL, S et al. 'Perilipins 2 and 3 lack a carboxy-terminal domain present in perilipin 1 involved in sequestering ABHD5 and suppressing basal lipolysis'; 24 June 2014, Proceedings of the National Academy of Sciences; Volume 111, Number 25, pages 9163-9168; abstract; page 9163, paragraphs [2]-[4]; page 9164, paragraphs [5]-[7]	1-3
Y	US 2004/0018221 A1 (BHAGWAT, D et al.) 29 January 2004; paragraphs [0008]-[0009], [0011]	5
Y	WO 2007/147370 A1 (UNIVERZITA KARLOVA V PRAZE FARMACEUTICKA FAKULTA V HRADCI KARLOVE) 27 December 2007; abstract; page 3, paragraph [3]	6

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 October 2016 (31.10.2016)

Date of mailing of the international search report

07 DEC 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774