



- (51) International Patent Classification:
C12N 15/113 (2010.01) C07H 21/02 (2006.01)
A61K 48/00 (2006.01)
- (21) International Application Number:
PCT/US2013/037579
- (22) International Filing Date:
22 April 2013 (22.04.2013)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/636,059 20 April 2012 (20.04.2012) US
61/681,750 10 August 2012 (10.08.2012) US
61/782,838 14 March 2013 (14.03.2013) US
- (71) Applicant: APTAMIR THERAPEUTICS, INC.
[US/US]; 484 Terracina Way, Naples, Florida 34119 (US).
- (72) Inventor: THIBONNIER, Marc; 484 Terracina Way,
Naples, Florida 34119 (US).
- (74) Agents: WILSON, Mark, B. et al.; FULBRIGHT & JA-
WORSKI LLP, 98 San Jacinto Blvd., Suite 1100, Austin,
TX 78701 (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, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, 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, 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, ML, MR, NE, SN, TD, TG).

Published:
— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: MIRNA MODULATORS OF THERMOGENESIS

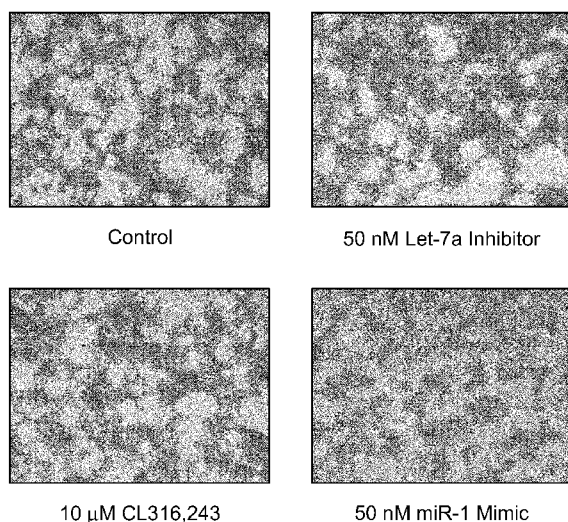


FIG. 22

(57) Abstract: Provided are novel methods and compositions for the modulation of thermogenesis. Such methods are particularly advantageous in that they allow for the reduction of body fat in a subject without the subject having to adjust their caloric intake through dieting, modify their physical activity or undergo bariatric surgery. Accordingly, the methods of the invention are particularly useful for treating or preventing obesity. Also provided are methods of screening for novel agents that modulate the activity of thermogenic regulators.

WO 2013/159091 A3

(88) Date of publication of the international search report:
30 April 2015

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/37579

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113; A61K 48/00; C07H 21/02 (2013.01)

USPC - 514/44A; 536/24.1, 24.5

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): C12N 15/113, 11, 00, 5/00, 02; A61K 48/00; C07H 21/02; A61P 3/00, 21/00 (2013.01)

USPC: 514/44A, 44R, 43, 42, 23, 1; 435/375, 325; 536/23.1, 22.1, 18.7, 1.11

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)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; DialogPRO; PubMed; 'UCP1, 'UCP2, 'UCP3, uncoupled, protein, mitochondria*, thermogenin, 'miRNA, 'microRNA, 'micro, 'RNA, 'mir, hsa*mir*, thermogenesis, agomir, antagomir, anti*mir, blockmir, adipocyte, adipogenesis, pre*adipocyte, rosiglitazone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/079263 A2 (COLLARD J et al.), June 30, 2011; abstract; paragraphs [0030], [0060], [0072], [00105], [00119], [00120], [00178]-[00180]	1-7, 8/1-8/7
X --- Y	CHEN, X et al. RNA-Mediated Regulation And Noncoding RNAs: In Vitro Evidence Suggests That miR-133a-mediated Regulation Of Uncoupling Protein 2 (UCP2) Is An Indispensable Step In Myogenic Differentiation. The Journal of Biological Chemistry. 2009, Vol. 284, pp 5362-5369; page 5362, column 2, paragraph 2 to page 5363, column 1, paragraph 1; page 5364, column 1, paragraph 3.	30-32 ----- 35/30-35/32, 36/35/30-36/35/32
X --- Y --- A	ALEXANDER, R et al. MicroRNAs In Adipogenesis And As Therapeutic Targets For Obesity. Expert Opinion in Therapeutic Targets. May 2011, Vol. 15, No. 5, pp 623-636 (Author Manuscript, October 7, 2011, pp 1-21); page 4, section 2.1, paragraph 1; page 9, section 3.2, paragraph 3 to page 10, section 3.2, paragraph 2.	30, 33 ----- 35/33, 36/35/33, 62-65, 69-79 ----- 34, 35/34, 36/35/34
Y	WO 2010/108126 A2 (MENDLEIN, JD et al.), September 23, 2010; page 133, line 3 to page 135, line 36; page 143, lines 20-27; page 145 line 14 to page 147, line 20; page 264, lines 24-31; page 267, line 20 to page 268, line 5; page 276, lines 10-15; page 321, line 30 to page 322, line 2.	35/30-35/33, 36/35/30-36/35/33, 52-54, 61-79

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 18 October 2013 (18.10.2013)	Date of mailing of the international search report 25 OCT 2013
---	--

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. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
---	--

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 9-25, 37-43
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please See Supplemental Page-

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-8, 30-36 and 44-79

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application no.

PCT/US13/37579

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN, T et al. MicroRNA let-7 Regulates 3T3-L1 Adipogenesis. <i>Molecular Endocrinology</i> , 2009, Vol. 23, pp 925 to 931; page 928, column 1, paragraph 1; 929, column 2, paragraph 1; page 930, column 2, paragraph 9 to page 931, column 1, paragraph 1.	55 -----
Y		56-61
Y	WO 2011/138457 A1 (SCHEIDELER, M et al.), November 10, 2011; abstract; page 14, line 32 to page 15, line 21; page 16, lines 20-33; page 46, lines 1-10	66-68
Y	WO 2012/007725 A2 (CHOO, Y et al.), January 19, 2012; page 17, lines 23-33; page 19, lines 1-6; page 23, lines 13-20; page 27, lines 25-28; page 47, lines 1-14; page 47, lines 22-27	53, 56-60
X	US 2011/0224286 A1 (YU, X et al.), September 15, 2011; paragraphs [0081], [0082], [0084], [0104], [0105], [0192]	44-51
Y	ZARAGOSI, LE et al. Small RNA Sequencing Reveals miR-642-3p As A Novel Adipocyte-Specific microRNA And miR-30 As A Key Regulator Of Human Adipogenesis. <i>Genome Biology</i> . 2011, Vol. 12, pp 1-13; page 2, column 2, paragraph 4; figure 2, Table 1.	52-54
A	RANTALAINEN, M et al. MicroRNA Expression In Abdominal And Gluteal Adipose Tissue Is Associated With mRNA Expression Levels And Partly Genetically Driven. <i>PLoS ONE</i> . 2011, Vol. 6, No. 11, pp 1-12; e27338. doi:10.1371/journal.pone.0027338; page 2, column 2, paragraph 3; page 8, column 1, paragraph 6-column 2, paragraph 1.	34, 35/34, 36/35/34

-Continued from Box No. III: Observations Where Unity Of Invention Is Lacking

Group I: Claims 1-8, 30-36, 44-79

Group II: Claims 26-29

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8, 30-36 and 44-79 are directed toward a method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with a miRNA agent that modulates activity of at least one mitochondrial uncoupler; a method of modulating thermogenesis in a tissue, the method comprising contacting the tissue with a miRNA agent that modulates activity of at least one mitochondrial uncoupler; a method of treating obesity in a human subject in need of treatment thereof, the method comprising administering to the human subject an effective amount of a miRNA agent that modulates activity or expression of at least one mitochondrial uncoupler; an agomir or antagomir that modulates the activity of at least one thermogenic regulator in a cell; a pharmaceutical composition comprising two or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of inducing pre-adipocytes to differentiate into adipocytes comprising administering to a population of pre-adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of decreasing the lipid content of adipocytes comprising administering to a population of adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method for increasing insulin sensitivity in a subject in need thereof comprising administering to the subject one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir and hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of increasing expression or activity of one or more uncoupling proteins in a cell comprising administering to the cell one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir; a method of causing fat loss in a subject in need thereof comprising administering to the subject one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir; use of an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 in the manufacture of a medicament for the treatment of obesity; and a composition comprising an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 for the treatment of obesity.

Group II: Claims 26-29 are directed toward a method of screening for a miRNA agent that modulates thermogenesis, the method comprising: a) providing an indicator cell comprising a human genome; b) contacting the indicator cell with a test miRNA agent; and c) determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with a miRNA agent that modulates activity of at least one mitochondrial uncoupler, which is not present in Group II; Group II having special technical features including a method of screening for a miRNA agent that modulates thermogenesis, the method comprising: a) providing an indicator cell comprising a human genome; b) contacting the indicator cell with a test miRNA agent; and c) determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis.

Groups I-II share the technical features including a method comprising a miRNA agent that modulates thermogenesis, the method comprising: a miRNA agent that modulates thermogenesis.

However, these shared technical features are previously disclosed by WO 2011/079263 A2 to Collard, et al. (hereinafter 'Collard'). Collard discloses a method (Claim 1) comprising a miRNA agent (paragraphs [00105], [00107]) that modulates thermogenesis (paragraph [0060]), the method (Claim 1) comprising: a miRNA agent (paragraphs [00105], [00107]) that modulates thermogenesis (paragraph [0060]).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Collard reference, unity of invention is lacking.