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(54) Title: COMPOUNDS AND METHODS FOR MODULATING APOLIPOPROTEIN C-III EXPRESSION FOR IMPROVING A DIABETIC PROFILE

(57) Abstract: Provided herein are improved compounds, compositions and associated methods for reducing expression of ApoCIII mRNA and protein in a subject having, or at risk of having, diabetes. Also provided herein are compounds, compositions and associated methods improving peripheral insulin sensitivity, a lipid profile and a diabetes profile as well as reducing free fatty acids and intra-muscular triglyceride deposition.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/43723

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12N 15/11, C07H 21/04, C12N 5/00 (2014.01) CPC - C12N 15/113, A61K 38/00, C12N 2310/321 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 15/11, C07H 21/04, C12N 5/00 (2014.01) CPC - C12N 15/113, A61K 38/00, C12N 2310/321 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 514/44A; 435/375, 536/24.5 (text search, term limited) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WEST; Google Scholar; PatBase search terms - ApoCIII, apo-CIII, apoc-III, ApoC3, Apolipoprotein C-III, Apolipoprotein C-3, Apolipoprotein C3, Apolipoprotein CIII, HALP2, apo-C3, apoc-3, antisen*, mirna, sirna, dsrna, shrna, rna, silenc*, insulin, sensitiv*, resisten*, diabetes, VLDL, ldl, hdl, fatty acids														
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>WO 2012/149495 A1 (MULLICK et al.) 01 November 2012 (01.11.2012) p. 7, ln 5-30; p. 11, ln 9-14; p. 19, ln 3-10; p. 20, ln 17-24; p. 21, ln 5-10; p. 26, ln 30-31; p. p. 61, p. 63; p. 67-68; p. 97, ln 8-15; SEQ ID NO: 3; Table 16.</td> <td>1, 2, 4, 5, 6/(1,2,4,5), 18</td> </tr> <tr> <td>A</td> <td>US 2011/0060030 A1 (CROOKE et al.) 10 March 2011 (10.03.2011) para [0272]; Table 16; SEQ ID NO: 87.</td> <td>1</td> </tr> <tr> <td>A</td> <td>Samuel et al. "Mechanisms for insulin resistance: common threads and missing links." Cell 148.5 (2012): 852-871. p. 855, para 3; p. 853, para 2.</td> <td>1</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	WO 2012/149495 A1 (MULLICK et al.) 01 November 2012 (01.11.2012) p. 7, ln 5-30; p. 11, ln 9-14; p. 19, ln 3-10; p. 20, ln 17-24; p. 21, ln 5-10; p. 26, ln 30-31; p. p. 61, p. 63; p. 67-68; p. 97, ln 8-15; SEQ ID NO: 3; Table 16.	1, 2, 4, 5, 6/(1,2,4,5), 18	A	US 2011/0060030 A1 (CROOKE et al.) 10 March 2011 (10.03.2011) para [0272]; Table 16; SEQ ID NO: 87.	1	A	Samuel et al. "Mechanisms for insulin resistance: common threads and missing links." Cell 148.5 (2012): 852-871. p. 855, para 3; p. 853, para 2.	1
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☐ Further documents are listed in the continuation of Box C. ☐														
* 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 07 January 2015 (07.01.2015)		Date of mailing of the international search report 06 FEB 2015												
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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774												

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/43723

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.: 7-17, 20-27
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:
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, 2, 4, 5, 6(in part) and 18, directed to a method of improving peripheral insulin sensitivity in a subject.

Group II, claims 3 and 6(in part), directed to a method of reducing intramyocellular triglyceride deposition.

Group III, claims 19, directed to a composition.

The inventions listed as Groups I-III 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:

--continued on first extra sheet attached hereto--

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.:
1, 2, 4, 5, 6(in part) and 18

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.

continuation of Box III: Observations where unity of invention is lacking

Special technical features

Group I has the special technical feature of a method of improving peripheral insulin sensitivity/a diabetes profile/a lipid profile, wherein insulin sensitivity (index), glucose disposal rate, glucose MCR, glucose and metabolism:insulin ratio is improved, that is not required by Group II.

Group II has the special technical feature of a method of reducing intramyocellular triglyceride deposition, that is not required by Group I.

Common technical features:

Groups I (a method of improving peripheral insulin sensitivity) and II (a method of reducing intramyocellular triglyceride deposition) are related to Group III (a compound for use in a subject to improve peripheral insulin sensitivity and/or reduce intramyocellular triglyceride deposition), and share the common technical feature of a compound comprising an ApoCIII specific antisense oligonucleotide for use in a subject to: improve peripheral insulin sensitivity, lower free fatty acids, reduce intramyocellular triglyceride deposition, improve a lipid profile; and/or improve a diabetic profile. Groups I and II further share the common feature of a therapeutic method comprising, selecting a subject with moderately controlled type II diabetes and administering a compound comprising an antisense oligonucleotide 12-30 nucleobases in length targeting ApoCIII, thereby having a therapeutic effect. However, these shared technical features do not represent a contribution over prior art, because these shared technical features are obviated by US 2011/0060030 A1 to Crooke et al. (hereinafter Crooke), in view of the article by Samuel entitled 'Mechanisms for insulin resistance: common threads and missing links', (hereinafter Samuel).

Crooke teaches a compound comprising an ApoCIII specific antisense oligonucleotide for use in a subject to lower free fatty acids or improve a lipid profile or improve a diabetic profile (para [0042]-[0043], [0272]-[0273] 'Effects of Antisense Inhibition of Rat Apolipoprotein C-III In Vivo: Blood Lipids...to significantly reduced circulating triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol in rats. Furthermore, these animals exhibited reduced expression of apolipoprotein C-III mRNA in liver following treatment with ISIS 340982'; Table 8; Table 16: free fatty acids 0.57 mEq/L at week 0 and 0.53 mEq/L at week 2 with 100mg/kg ISIS 340982; [0078] 'apolipoprotein C-III inhibitors are useful in the treatment of hypertriglyceridemia, abnormal lipid metabolism').

Crooke further teaches a therapeutic method (para [0015]), comprising selecting a subject with type II diabetes (para [0015], [0078] 'such a compound that reduces levels of apolipoprotein C-III is useful to prevent morbidity and mortality for subjects with cardiac-related disorders...reduction in apolipoprotein C-III can result in a reduction in the serum levels of cholesterol, triglycerides, and glucose. Thus, apolipoprotein C-III inhibitors are useful in the treatment of hypertriglyceridemia, abnormal lipid metabolism, abnormal cholesterol metabolism, atherosclerosis, hyperlipidemia, diabetes, including Type 2 diabetes, obesity, cardiovascular disease, coronary artery disease, among other disorders relating to abnormal metabolism or otherwise') and administering a compound comprising an antisense oligonucleotide 12-30 nucleobases in length targeting ApoCIII (para [0013], [0015]-[0016]), thereby having a therapeutic effect (para [0015], [0078]).

Crooke does not specifically teach the therapeutic method comprises improving peripheral insulin sensitivity or reducing intramyocellular triglyceride deposition, or wherein the type II diabetes is moderately controlled. However, Crooke does teach treatment of any disorders associated with apolipoprotein C-III expression (para [0015] 'disease or condition associated with expression of apolipoprotein C-III'; [0078]).

Samuel teaches insulin sensitivity is directly associated with apolipoprotein C-III expression (p. 855, para 3 'ApoC3 can inhibit LpL activity and limit peripheral fat uptake, thereby promoting postprandial hyperlipidemia. Though transgenic mice that overexpress ApoC3 (ApoC3 tg) have marked hypertriglyceridemia, hepatic lipid content in regular chow-fed mice is not different than wild-type mice fed the same diet (Lee et al., 2011b). However, when placed on a high-fat diet, ApoC3 tg mice develop hepatic steatosis with diacylglycerol accumulation, PKC α activation, and hepatic insulin resistance...Thus, as with the ApoC3 tg mice, when individuals carrying polymorphisms in ApoC3 exist in ??toxic environments?? (Novak and Brownell, 2011), they are prone to NAFLD and hepatic insulin resistance.'), and intramyocellular triglyceride content is a strong predictor of insulin resistance (p. 853, para 2 'intramyocellular triglyceride content is a far stronger predictor of muscle insulin resistance than circulating fatty acids (Krssak et al., 1999), suggesting that intramyocellular lipids may cause muscle insulin resistance'). It would have been obvious to one of ordinary skill in the art to have applied the therapeutic compositions and methods taught by Crooke for improving peripheral insulin sensitivity and/or reducing intramyocellular triglyceride deposition, wherein the type II diabetes is moderately controlled, since one of ordinary skill in the art would have readily appreciated that since insulin sensitivity and related intramyocellular triglyceride content have been shown to be directly associated with apolipoprotein C-III expression (Samuel), inhibition of apolipoprotein C-III expression via the methods and compositions of Crooke would improve insulin sensitivity and reduce associated intramyocellular triglyceride deposition in patients having insulin sensitivity, including those where type II diabetes is moderately controlled, and further readily appreciated application to different types of insulin sensitivity including peripheral insulin sensitivity during the ordinary course of experimentation, since there is a common underlying lipid regulation involved, and amelioration of diabetes in any stage/level of control would be therapeutically beneficial.

Therefore, Groups I-III lack unity of invention under PCT Rule 13.

Note: Claims 7-17 and 20-27 have been found to be unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Note: Claim 13, as drafted, is a dependent claim of claim 14. Since a claim cannot depend on a later claim, claim 13 is reconstructed as a dependent claim of claim 12.