

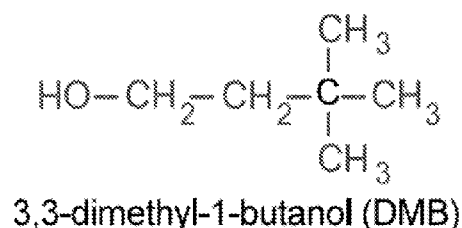
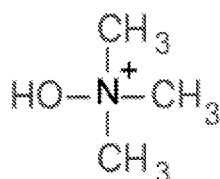
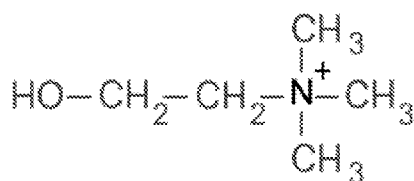


- (51) International Patent Classification:
A23L 1/30 (2006.01) *A61K 31/4164* (2006.01)
A61K 31/045 (2006.01)
- (21) International Application Number: PCT/US2013/045197
- (22) International Filing Date: 11 June 2013 (11.06.2013)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 61/658,208 11 June 2012 (11.06.2012) US
- (71) Applicant: THE CLEVELAND CLINIC FOUNDATION [US/US]; 9500 Euclid Avenue, Cleveland, Ohio 44195 (US).
- (72) Inventors; and
(71) Applicants : HAZEN, Stanley L. [US/US]; 31650 Gates Mills Blvd, Pepper Pike, Ohio 44124 (US). LEVISON, Bruce [US/US]; 1534 Jennifer Drive, Twinsburg, Ohio 44087 (US). WANG, Zeneng [CN/US]; 2988 Green Road, Shaker Heights, Ohio 44122 (US).
- (74) Agent: BOND, Jason R.; 2275 Deming Way, Ste 310, Middleton, Wisconsin 53562 (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,

[Continued on next page]

(54) Title: TREATMENT AND PREVENTION OF CARDIOVASCULAR DISEASE AND THROMBOSIS

FIG. 1



(57) Abstract: Provided herein are compositions for the treatment and/or prevention of cardiovascular disease (CVD), and methods of application and use thereof. In particular, the present invention provides treatment and/or prevention of cardiovascular disease with compounds that inhibit the production of TMA in the gut, such as 3,3-dimethyl-1-butanol (DMB) or other compounds represented by Formula I or as shown in Figures 20-23.





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, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

20 March 2014

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/45197

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/30; A61K 31/045, 31/4164 (2013.01)

USPC - 424/451

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) - A23L 1/30; A61K 31/045, 31/4164 (2013.01)

USPC - 424/451; 514/253.08, 724

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 Scholar; DialogPro; IP.com; N,N-dimethylethanolamine, dimethylethanolamine, cardiovascular, thrombosis, TMAO, platelet aggregation, probiotic, food, beverage*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	WO 2001/010428 A2 (YAVIN, E et al.) 15 February 2001; abstract; page 3, lines 4-6; claims 9, 11	12 — 1-4
X	US 2010/0080863 A1 (SOMMERFELD, A et al.) 01 April 2010; abstract	20
Y	US 7,504,497 B2 (DOUGLASS, III, JG et al.) 17 March 2009; abstract; column 29, lines 28-30; claim 10	1, 3
Y	WANG, Z et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature, Vol. 472, No. 7341, 07 April 2011, pp. 57-63, National Institutes of Health Public Access, Author Manuscript, 07 October 2011, pp. 1-22 [online], [retrieved on 2013-10-23]. Retrieved from the Internet <URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3086762/pdf/nihms-271797.pdf > <DOI: 10.1038/nature09922>; page 5, lines 15-16, last paragraph; page 6, first paragraph; page 8, third paragraph	1-2, 4
A	WO 2011/019685 A2 (QUAY, SC) 17 February 2011; abstract; page 1, last paragraph; page 4, first paragraph	1, 3

☐ 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

17 January 2014 (17.01.2014)

Date of mailing of the international search report

24 JAN 2014

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/45197

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.:
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.

Groups I+: Claims 1-20; where the compound is selected from the group consisting of: DMEA, MEA, and EA is directed toward a method for the treatment and/or prevention of cardiovascular disease and/or thrombosis, and a food or beverage

Exemplary Election: where the compound is selected from the group consisting of: trimethylsilyl ethanol, P,P,P-trimethyl ethanolphosphine, or a compound of Formula I.

-***-Continued Within the Next Supplemental Box-***-

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.:
Claims 1-4, 12 and 20

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.

-Continued from Box No. III: Observations where unity of invention is lacking-

Group I+ will be searched to the extent that it encompasses the first named invention (a composition comprising a compound selected from the group consisting of: N,N-dimethylethanolamine (DMEA), N-methylethanolamine (MEA), and ethanolamine (EA), and a food or beverage), without fee. It is believed that Claims 1-4, 12 and 20 encompass this first named invention. Applicants must indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group will result in only the first claimed invention to be searched/examined.

The exemplary election for the additional compounds would be: where the compound is selected from the group consisting of: trimethylsilyl ethanol, P,P,P-trimethyl ethanolphosphine, or a compound of Formula I.

Compounds of the composition of i) share common elements and have the shared features, including a method for the treatment and/or prevention of cardiovascular disease and/or thrombosis comprising: a) identifying a subject as having increased platelet aggregation and/or elevated TMAO levels, and b) administering to said subject: i) a composition comprising the compound; and/or ii) a composition comprising a compound represented by Formula I.

However, these common elements are previously disclosed by WO 2001/10428 A2 to Yavin, et al. (hereinafter 'Yavin') in view of US 7,504,497 B2 to Douglass, III, et al. (hereinafter 'Douglass'). Yavin discloses a method for the treatment and/or prevention of cardiovascular disease and/or thrombosis (preventing cell death induced by oxidative stress, in particular for the alleviation of ischemic stroke, thromboembolic or hemorrhagic stroke and cardiac arrest; abstract) comprising: b) administering to said subject (composition is formulated for oral administration; page 3, lines 10-12): i) a composition comprising the compound (in one preferred embodiment, the active ingredient of the composition is N,N'-dimethylethanolamine; page 3, lines 10-12); and/or ii) a composition comprising a compound represented by Formula I. Yavin does not disclose a) identifying a subject as having increased platelet aggregation and/or elevated TMAO levels. However, Douglass discloses a) identifying a subject as having increased platelet aggregation and/or elevated TMAO levels (method of preventing or treating diseases or conditions associated with platelet aggregation, comprising administering to a subject in need thereof an assay for the measurement of the aggregation; abstract; column 29, lines 24-30; claim 10). It would have been obvious to one skilled in the art, at the time the invention was made, to have modified the method, as previously disclosed by Yavin, in order to have provided for a) identifying a subject as having increased platelet aggregation and/or elevated TMAO levels, as previously disclosed by Douglass, for providing for a more effective and focused treatment method. Additionally, where Yavin discloses the method for the treatment of thromboembolic (Yavin; abstract), said method comprising a composition comprising N,N-dimethylethanolamine (Yavin; page 3, lines 10-12), and where Douglass discloses a method for treating thrombosis (Douglass; abstract), said method including a means for measuring platelet aggregation (Douglass; column 29, lines 24-30), the modification of providing for the identification of increased platelet aggregation and/or elevated TMAO levels could have been readily achieved through routine experimentation and testing, provided the previous disclosures of Yavin and Douglass.

Since none of the special technical features of the Groups I+ inventions are found in more than one of the inventions, and since all of the shared technical features and shared structural elements between the listed compounds are previously disclosed by Yavin, in view of Douglass, unity of invention is lacking.