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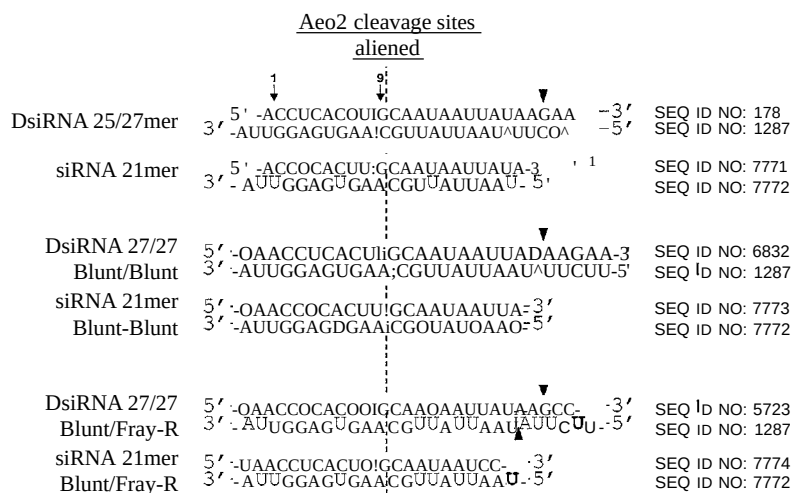
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(54) **Title:** METHODS AND COMPOSITIONS FOR THE SPECIFIC INHIBITION OF BETA-CATENIN BY DOUBLE-STRANDED RNA

Figure 1

(57) **Abstract:** This invention relates to compounds, compositions, and methods useful for reducing β -catenin target RNA and protein levels via use of dsRNAs, e.g., Dicer substrate siRNA (DsiRNA) agents.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/42820

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C 12N 15/11; C07H 21/04 (201.1.01)
 USPC - 514/44A; 536/24.5

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 15/11; C07H 21/04 (201.1.01)
 USPC - 514/44A; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 514/44R

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google Scholar: Ctnnbl, B, beta, catenin, Single, terminS, mirna, rna, sirna, sina, microrna, rna, dsrna, NucleotidS, pairs, duplex, duplexS, complemenS, overhang, mammS, rna, inhibS, silenS, interferS, double, strand, length, long, longer, modifS, inmodifS, methyl. GenCore 6.3: SEQ ID NOs: 8, 1117, 2226

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sun, et al. Asymmetric RNA duplexes mediate RNA interference in mammalian cells. Nature Biotechnology 2008, 26(12): 1379-1382; pg 1379, 2nd and 3rd para; pg 1380, 2nd para; pg 1381, 2nd para; Fig. 1; Fig. 3a; abstract	1, 3-51, 54-61, 63, 64
Y	US 2009/0176725 A1 (MORRISSEY, et al.) 09 July 2009 (09.07.2009) para [0036]-[0037]; [0040]-[0041]; [0048]; [0056]; [0065]; [0085]; [0090]; [0119]; [0124]; [0126]; [0141]; [0146]-[0147]; [0154]; [0155]-[0327]; [0384]; [0398]; [0454]; [0495]; [0587]; [0645]	1, 3-52, 54-61, 63, 64, 66
Y	US 2003/0064384 A1 (HUNG, et al.) 03 April 2003 (03.04.2003) para [0028]; SEQ ID NO: 12	1, 3-52, 54-61, 63, 64, 66
Y	US 2009/0203137 A1 (WORM) 13 August 2009 (13.08.2009) para [0009]; [0013]; [0017]; [0020]-[0021]; [0023]; [0028]-[0029]; [0031]-[0032]; [0037]; [0047]-[0051]; [0058]; [0060]; Fig. 4	35-44, 52, 54-61, 66
Y	WO 2007/031091 A2 (FLUITER, et al.) 22 March 2007 (22.03.2007) pg 44, 2nd para	40
A	US 2004/0175703 A1 (KREUTZER, et al.) 09 September 2004 (09.09.2004) para [0008]; [0009]; [0058]; [0059]	1, 3-52, 54-61, 63, 64, 66



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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/42820

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.

Group I+: claims 1-69, drawn to an isolated double stranded ribonucleic acid (dsRNA) comprising first and second nucleic acid strands and a duplex region of at least 25 base pairs, wherein said first strand is 25-34 nucleotides in length and said second strand of said dsRNA is 26-35 nucleotides in length and comprises 1-5 single-stranded nucleotides at its 3' terminus, wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence selected from the group consisting of SEQ ID NOS: 2226-3334 along at least 15 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell. The first invention is restricted to SEQ ID NO: 8, 1117, and 2226. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. [NOTE: Claims 2, 53, 65, 67-69 were excluded from the search, because they are drawn to a non-elected subject matter.]

-----continued on first blank 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, 3-52, 54-61, 63, 64 and 66, restricted to SEQ ID NOS: 8, 1117, 2226

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 No. III: Observations where unity of invention is lacking

Group II: claims 70-160, drawn to an isolated dsRNA comprising first and second nucleic acid strands, wherein said first strand is 15-35 nucleotides in length and said second strand of said dsRNA is 19-35 nucleotides in length, wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence selected from Table 18 along at least 19 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell.

The inventions listed as Groups I+ and 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 inventions of Group I+ do not include the inventive concept of an isolated dsRNA comprising first and second nucleic acid strands, wherein said first strand is 15-35 nucleotides in length and said second strand of said dsRNA is 19-35 nucleotides in length, wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence selected from the group consisting of SEQ ID NOs: 2226-3334 along at least 15 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell, as required by Group II.

The inventions of Group II do not include the inventive concept of an isolated dsRNA comprising first and second nucleic acid strands and a duplex region of at least 25 base pairs, wherein said first strand is 25-34 nucleotides in length and said second strand of said dsRNA is 26-35 nucleotides in length and comprises 1-5 single-stranded nucleotides at its 3' terminus, wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence along at least 15 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell, as required by Group I+.

The inventions of Group I+ share the technical feature of an isolated dsRNA comprising first and second nucleic acid strands and a duplex region of at least 25 base pairs, wherein said first strand is 25-34 nucleotides in length and said second strand of said dsRNA is 26-35 nucleotides in length and comprises 1-5 single-stranded nucleotides at its 3' terminus, wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence along at least 15 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell. This shared technical feature does not represent a contribution over prior art as being obvious over the article titled 'Asymmetric RNA duplexes mediate RNA interference in mammalian cells' by Sun et al. (hereinafter "Sun") in view of US 2004/0175703 A1 to Kreutzer et al. (hereinafter "Kreutzer") as follows:

Sun teaches an isolated double stranded ribonucleic acid (dsRNA) comprising first and second nucleic acid strands and a duplex region (p. 1379, 3rd para and Fig. 1a, 'We designed asymmetric RNA duplexes of various lengths with overhangs at the 3' and 5' ends of the antisense strand to target CTNNB1 (b-catenin) (Fig. 1a and Supplementary Fig. 1 online), which has been silenced using standard siRNA22. All siRNAs against CTNNB1 were designed to match the same 19-nt sequence targeted by the siRNA (Fig. 1a), but with an 5' antisense overhang containing two nontargeting nucleotides', wherein said second strand of said dsRNA comprises 1-5 single-stranded nucleotides at its 3' terminus (p. 1379, 3rd para and Fig. 1a), wherein said second oligonucleotide strand is sufficiently complementary to a target beta-catenin cDNA sequence along at least 15 nucleotides of said second oligonucleotide strand length to reduce beta-catenin target gene expression when said double stranded nucleic acid is introduced into a mammalian cell (p. 1379, 3rd para and Fig. 1):

Sun does not specifically teach the duplex region is of at least 25 base pairs, wherein said first strand is 25-34 nucleotides in length and said second strand of said dsRNA is 26-35 nucleotides in length. However, Sun does teach testing dsRNAs of various lengths including dsRNAs having 19 nucleotide duplexes, and strands of 21 nucleotides (p. 1379, 3rd para and Fig. 1a).

Kreutzer teaches dsRNAs comprising first and second nucleic acid strands and a duplex region having up to about 49 nucleotides (para [0008] and [0048]), wherein said first and second strands are 19-49 nucleotides in length (para [0008]-[0009]). It would have been obvious to one of ordinary skill in the art to have provided an isolated dsRNA as claimed, wherein the duplex region is of at least 25 base pairs, wherein said first strand is 25-34 nucleotides in length and said second strand of said dsRNA is 26-35 nucleotides in length, because of Sun teaches testing dsRNAs of various lengths for optimization including dsRNAs having 19 nucleotide duplexes, and strands of 21 nucleotides (p. 1379, 3rd para and Fig. 1a), and further because Kreutzer teaches dsRNAs effective for target gene silencing comprising first and second nucleic acid strands and a duplex region having up to about 49 nucleotides (para [0008] and [0048]), wherein said first and second strands are 19-49 nucleotides in length (para [0008]-[0009]), since a skilled artisan would have readily appreciated application of the dsRNA strand lengths taught by Kreutzer to beta catenin dsRNAs taught by Sun during the ordinary course of experimentation, since Kreutzer teaches this length range of dsRNAs to be effective for target gene silencing, and could therefore generate dsRNAs effective at silencing beta catenin. As said dsRNA would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I+ is a specific dsRNA targeting a specific nucleic acid recited therein. The inventions do not share a special technical feature, because US 2003/0064384 A1 to Hung et al. (hereinafter "Hung") teaches a beta-catenin DNA sequence comprising SEQ ID NO: 2226 (para [0028] and SEQ ID NO: 12). Therefore, no significant structural similarities can readily be ascertained among the claimed sequences. Without a shared special technical feature, the inventions lack unity with one another.

Thus, there is no unity under PCT Rule.