Journal of Advances in Medical and Pharmaceutical Sciences
5(2): 1-10, 2016, Article no.JAMPS.22023
ISSN: 2394-1111

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Rescuing Abandoned Molecules as Nav1.7 and PCSK9 Inhibitors

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GA, LB and SG designed the study and wrote the protocol. Authors LB and GA collected all data, and wrote the first draft of the manuscript. Author GA did the literature search. Authors GA, LB, SG, GR, LS, MNM, MAS, AM and VP wrote part of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/22023
Editor(s):
(1) Esam Z. Dajani, President, IDDC Corporation, USA, Adjunct Professor of Medicine, Loyola University Chicago, USA.
Reviewers:
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(4) Khalid M. Khan, University of Karachi, Pakistan.
Complete Peer review History: http://sciencedomain.org/review-history/12115

Short Research Article

Received 14th September 2015
Accepted 6th October 2015
Published 6th November 2015

ABSTRACT

Academic institutions have plentiful of unexploited compounds. A cost-effective repositioning strategy from abandoned molecules coming from different research projects was applied. This strategy was based on the creation of a 2D-database then the biological assaying following adherence to the the Lilly OIDD (Open Innovation Drug Discovery) program by combining *in silico* methods and *in vitro* screening modules representing therapeutic areas of significant interest. The best results involve some molecules, that demonstrated to be active voltage-gated sodium ion channel Nav1.7 inhibitors. The inhibition of Nav1.7 is considered to be a potential mechanism for the treatment of chronic pain. Other findings refer to the inhibition of PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) synthesis, a convertase that plays a regulatory role in the metabolism of cholesterol. This work demonstrates that successful strategies can be established by

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mutual Academic-Industrial collaborations that can lead to the discovery and development of new drugs by a cost-effective approach by accessing complementary crucial skills and cutting-edge technologies, without costly investments to generate the highest quality data as efficiently as possible with small amounts of compound.
Extracting latent value from abandoned molecules from various academic research projects, might be the starting point for innovative and cost-saving opportunities for both Academia and Industry to accelerate discovery of new drugs and disease treatments.

Keywords: Drug repositioning; database building; cheminformatics; in vitro screening; Nav1.7; PCSK9.

1. INTRODUCTION

Nowadays the drug discovery process requires a considerable effort in terms of time, cost and failure risk. Drug development takes several years due to the requirements regarding safety, efficacy and quality. Moreover, the attrition rate is another relevant aspect to be considered during the main stages of the drug development process (discovery, lead identification-optimization, preclinical studies and clinical trials); hundreds of thousands of molecules are discarded before the final product is obtained.

In this context it becomes reasonable to find new strategies for optimizing the available resources. Admirable as it is, the drug discovery and development process is continuously undergoing changes and adjustments in search of further improvements in efficiency, productivity, and profitability. Academic-industrial partnerships allow for transdisciplinary collaborations as a powerful means to advance the drug discovery and development process. Academic collections can be an excellent source to rescue or reprofile molecules, exploiting at best low level of investment, reduced development timeline, recycling of resources [1]. New technologies as computational predictions, virtual screening and HTS (high-throughput screening) methods can be powerful tools for a drug-rescuing strategy. Integration and interaction by continuous communication improve the quality and makes faster the drug development. Overall pharmaceutical companies should collaborate with academic for *hits fishing* and *prioritizing*, with the advantage of increased chances to reduce costs as well share own research goals. Correctly and fastly measuring biological activity and pharmacokinetics is nowadays substantial for supporting drug discovery. This is not always possible for academic Institutions for all for novel biological targets.

Indeed the adherence to the Lilly OIDD program allowed us to evaluate our molecules through cheminformatics tools in the Lilly OIDD web platform [2], which allowed to identify molecules to be candidates for the *in vitro* screening as well through phenotypic and target-based assays modules which represent interesting therapeutic areas [3]. Our results show that drug rescuing by academic collections can be successful both in *reinventing* molecules to address a new therapeutic actions and finding business opportunities.

The Lilly OIDD (Open Innovation Drug Discovery) screening module based on Nav1.7 aims to find a selective antagonist for this sodium channel which is expressed primarily in the peripheral sensory neurons and sympathetic ganglia. Selective antagonist of Nav1.7 channel, may be a promising therapeutic approach in the treatment of chronic pain [4]. The Lilly OIDD (Open Innovation Drug Discovery) phenotypic screening module based on PCSK9 synthesis inhibition aims to identify compounds that inhibit PCSK9 transcription using assays based on HepG2 (human hepatoma) cell lines. This protein convertase, induces the degradation of LDLR (Low Density Lipoprotein Receptor). The inhibition of PCSK9 reduces plasma levels of LDL cholesterol, increasing the function of LDL receptors that mediate the endocytosis of LDL [5]. Therefore, compounds which inhibit the PCSK9 synthesis, represent a valid approach in the treatment of hypercholesterolemia and related diseases.

2. MATERIALS AND METHODS

Abandoned molecules coming from different research projects were submitted to Lilly OIDD program [2]. Although the dataset is quite small when compared to the whole collection, promising activities were found as sodium channels Nav1.7 and of PCSK9 synthesis inhibition.

2.1 Database Building

The database was built by molecules selected from papers published by groups of the Department of Drug Sciences of the University of Catania. The used filtering criteria for the selection were:

- Corresponding author/s from the Department of Drug Sciences of the University of Catania.
- Period 2002 – 2012.
- Origin (were selected only molecules synthetized in the Drug Sciences Department of Catania).

Finally, 38 publications were selected from which 627 molecules of interest were extracted. The drawing process was carried out on a desktop PC, CPU Intel® Core™ i7 960 3.20 GHz - RAM 16GB. Structures were sketched as 2D MDL Molfiles using Accelrys – AccelrysDraw 4.1 [6]. The main stereochemical variants for each compound were considered; therefore enantiomers and stereoisomers for molecules which have asymmetric carbons (R/S) and/or double bonds (E/Z) were also drawn, respectively. The creation of molecular libraries to build the database was carried out by Accelrys – Discovery Studio 3.5 Client [6]. Libraries were built as SDF files. Finally, we have created 38 libraries containing overall 627 molecular structures.

2.2 *In silico* Screening

In-silico screening was performed by the automated procedure "cheminformatics structure evaluation" [7] a sequence of algorithms written by Eli Lilly & C. *In silico* evaluation has been used to filter the large structures database to the subsequent *in-vitro* Screening (HTS). The overall cheminformatics processing was based on a set of rules for identifying potentially reactive or promising compounds [8], and to evaluate the innovativeness and properties of analyzed structures. Finally, the algorithms were used also for evaluating the "drug-likeness" of the structures [9]. Cheminformatics structure evaluation was performed in different sequential steps which were essentially based on: substructure searching tools, filters to discard pharmaceutically undesirable motifs, molecular similarity comparison and model predictions. The molecular structures were stored encrypted in the secured area of the Lilly OIDD website. The cheminformatics structure evaluation started with an automatic conversion of the structures into molecular fingerprints. Only the generated bit-strings passed to the Lilly internal network for the *in-silico* evaluation process. All the chemical structures submitted were totally encrypted, therefore the confidentiality of compounds was absolutely ensured.

The fingerprint generation for the molecules under study was performed through linear and circular path methods, then the fingerprints were combined with a small set of calculated molecular properties [7]. The similarity measures between two fingerprints were calculated using the Tanimoto coefficient also known as Jaccard coefficient [10]. The computational tools used during Lilly OIDD cheminformatics structure evaluation included also QSAR (quantitative structure-activity relationship) models based on Tanimoto kernel function. These models have been used in a virtual screening assessment on some specific targets of Lilly OIDD program.

2.3 *In-vitro* Screening

In-vitro evaluation was performed by the Open Innovation Drug Discovery Screening Panel. The high-throughput screening was performed in the laboratories of Eli Lilly and Company, Indiana – USA. The performed assays comprised several target-based and phenotypic modules. The flow of each module included primary, secondary and confirmatory assays with established thresholds for progressing during the module flow. The main details concerning targets, phenotypes as well the flow schemes about all modules of the *in-vitro* screening are available by the Lilly OIDD website [2]. The assays of Lilly OIDD program have been developed according with the Assay Guidance Manual available on the National Center for Biotechnology Information [11].

The Percentage stimulation and percentage inhibition were calculated by Equation (1) and Equation (2), respectively, using the maximum (Max) and minimum (Min) response conditions for each assay:

- Equation (1): Stimulation % = (Signal – Min / Max – Min) x 100
- Equation (2): Inhibition % = 1 – (Signal – Min / Max – Min) x 100

The EC_{50} or IC_{50} of tested compounds has been determined using a standard four-parameter logistic and nonlinear regression analyses based

on the calculated percentage activation or inhibition values.

Concerning the target-based module Nav1.7, the assays have been performed on HEK-293 (Human Embryonic Kidney 293) cells, using the IonWorks® Quattro™ (IWQ) electrophysiology platform with automated patch clamp system.

The primary assays to evaluate the synthesis inhibition of PCSK9 have been performed on Human cell line: HepG2 (Hepatocellular carcinoma). The inhibition of non-specific secretion has been tested through a Metridia Luciferase Reporter Assay [11]. The secondary assays were based on enzyme-linked immunosorbent assays (ELISA) and have been performed on RPH (Primary Rat Hepatocyte) cells [11].

3. RESULTS AND DISCUSSION

627 compounds that were selected from 38 published works by research groups from the Drug Sciences Department, University of Catania were screened by chemoinformatics structure evaluation. 213 out of the 627 molecules passed the cheminformatics evaluation. 65 out of the 213 samples were tested by the Lilly OIDD *in-vitro* screening modules.

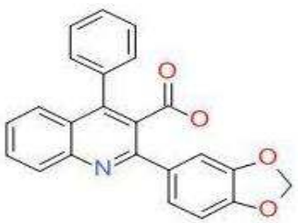
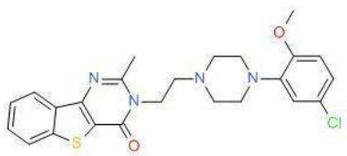
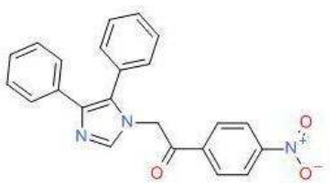
In the PCSK9 Screening module, 3 compounds showed an inhibition of PCSK9 synthesis during the primary assays on human liver hepatocellular carcinoma cell line (HepG2). Table 1 summarizes both the results of primary Single Point (SP) percentage inhibition assays performed in two different concentrations (5 µM - 50 µM) and values of IC_{50} obtained through primary Concentration Response Curve (CRC) assay. In the same table, the latter IC_{50} values of Metridia Luciferase Reporter assay (MLR) demonstrate how the mentioned compounds have not a relevant inhibition of non-specific secretion. Table 1 also summarizes the results of secondary Single Point (SP) assays that were performed on rat primary hippocampal cell line (RPH) in two different concentrations (20 µM - 100 µM) and twice duplicated for each concentration, R1 and R2. In the secondary assays, the compounds did not confirm the inhibition activity previously demonstrated on HepG2 cell line during primary assays. Due to the low values of percentage inhibition on RPH primary cell line, the mentioned compounds have not been accepted for further assays of the Lilly OIDD PCSK9 module.

The above-mentioned compounds (Fig. 1) that show an activity for the inhibition of PCSK9 synthesis were originally synthesized for different targets. Compound PV0235 belongs to a series of 2-substituted-4-aryl-3-quinolinecarboxylic acid derivatives which were originally synthesized as ligands of endothelin ET_A receptor [12]. Compound RG0222 shows the best value of IC_{50} as inhibitor of PCSK9 synthesis on HepG2 (Table 1). This compound was initially synthesized as selective ligand of α1 adrenoceptor [13]. Finally, compound SL0134 belongs to a series of imidazole-based ligands that were originally synthesized as inhibitors of nitric oxide synthase [14]. Even more interesting would be considered the similarity of some molecular substructures between PV0235 and the natural compound Berberine (Fig. 1), an alkaloid that suppress the PCSK9 transcription [15]. Further studies will be carried out to obtain compounds having a better PCSK9 synthesis inhibition profile.

The best results of our drug repositioning strategy involved the screening module based on the identification of selective Nav1.7 inhibitors. Six compounds demonstrated inhibition during primary assays on Nav1.7; the obtained values of Single Point (SP) percentage inhibition and values of IC_{50} are shown in Table 2. The Concentration Response Curve (CRC) assays used to calculate the values of IC_{50} were duplicated R1 and R2. All six compounds also demonstrated inhibition during the secondary assay that involved Nav1.5, a different subtype of voltage-gated sodium ion channel which is expressed primarily in the cardiac muscle. The IC_{50} values on Nav1.5 (Table 2), demonstrated a low selectivity of the compounds between the targets Nav1.7 and Nav1.5.

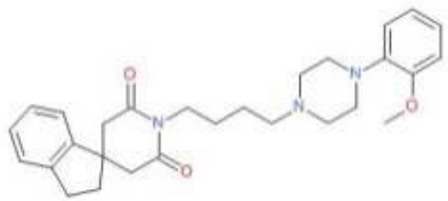
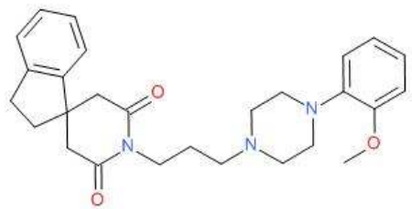
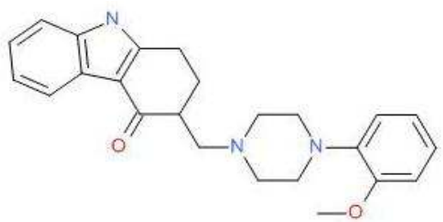
The best Nav1.7 inhibition activity has been demonstrated by the compounds RG0159, RG0158 and RG0321 (Fig. 2). These molecules were originally synthesized as selective ligands for α1-adrenoceptor subtypes [16,17]. RG0428 (Fig. 2) also belongs to a series of ligands for α1-adrenoceptor subtypes [18]. Compound SMA0132 (Fig. 2) belongs to a series of potent and selective 5-HT1A Serotonin receptor ligands [19]. MA0110RS (Fig. 2) that was initially synthesized as selective ligand of σ receptor [20], demonstrated the lowest inhibition activity on both Nav1.7 and Nav1.5 sodium channels.

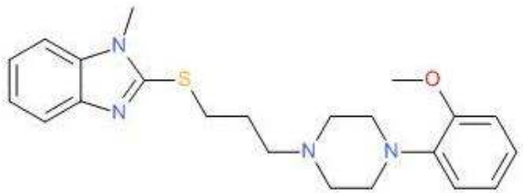
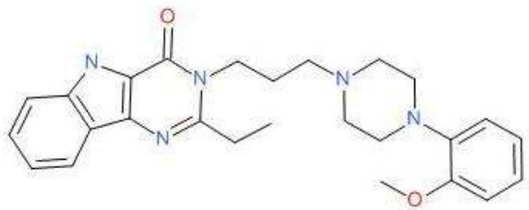
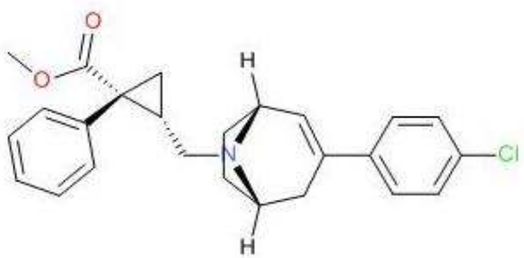
Table 1. PCSK9 screening module - Primary and secondary assays

Compound	Primary SP % inhib. (5 µM) HepG2	Primary SP % inhib. (50 µM) HepG2	Primary CRC IC_{50} (µM) HepG2	Primary MLR IC_{50} (µM) HepG2	Secondary SP % inhib. (20 µM) RPH (R1)	Secondary SP % inhib. (20 µM) RPH (R2)	Secondary SP % inhib. (100 µM) RPH (R1)	Secondary SP % inhib. (100 µM) RPH (R2)
PV0235 	6,6	107,4	4,163	> 50,0	3,613	- 4,145	13,25	10,34
RG0222 	5,6	71,3	0,1472	47,41	- 8,886	- 5,29	27,34	15,27
SL0134 	- 30,4	98,6	6,827	33,19	7, 494	7,538	82,78	94,48

SP % inhib. = Percentage of inhibition in the single point assay.
CRC IC_{50} = IC_{50} value in the concentration response curve assay.
MLR IC_{50} = IC_{50} value in the Metridia Luciferase Reporter assay to evaluate the inhibition of non-specific secretion.
HepG2 = Human liver hepatocellular carcinoma cell line.
RPH = Rat primary hippocampal cell line.
R1 = First run of the assay, R2 = Second run of the assay

Table 2. Nav1.7 screening module - Primary and secondary assays

Compound		Primary	Primary	Primary	Secondary	Secondary
		SP % inhib. (3 μM)	CRC IC_{50} (μM)	CRC IC_{50} (μM)	CRC IC_{50} (μM)	CRC IC_{50} (μM)
		hNav1.7	hNav1.7 (R1)	hNav1.7 (R2)	hNav1.5 (R1)	hNav1.5 (R2)
RG0159		98,9	0.4329	0.745	1.298	1.122
RG0158		91.8	1.49	1.007	2.395	2.168
RG0321		91.6	1.685	0.7944	3.291	1.096

Compound	Primary SP % inhib. (3 µM) hNav1.7	Primary CRC IC_{50} (µM) hNav1.7 (R1)	Primary CRC IC_{50} (µM) hNav1.7 (R2)	Secondary CRC IC_{50} (µM) hNav1.5 (R1)	Secondary CRC IC_{50} (µM) hNav1.5 (R2)
SMA0132 	69.6	4.014	1.718	1.639	1.998
RG0428 	60.7	2.145	1.768	1.862	1.871
MA0110RS 	60.2	3.507	4.117	5.86	4.362

SP % inhib. = Percentage of inhibition in the Single Point assay.
CRC IC_{50} = IC_{50} value in the Concentration Response Curve assay.
hNav1.7 = Human voltage-gated sodium ion channel subtype 1.7 [4]
hNav1.5 = Human voltage-gated sodium ion channel subtype 1.5 [4]
R1 = First run of the assay, R2 = Second run of the assay

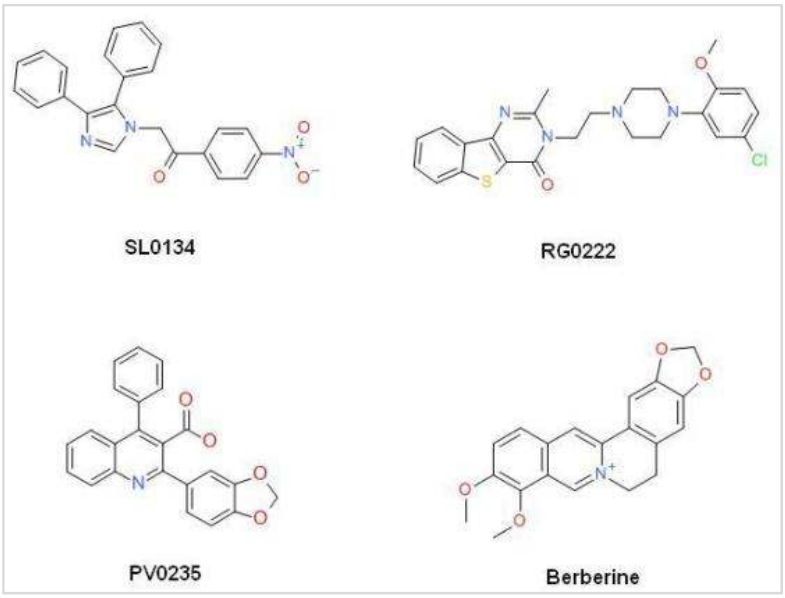


Fig. 1. Compounds under study that showed inhibition of PCSK9 synthesis; Berberine is a natural alkaloid that decreases PCSK9 expression

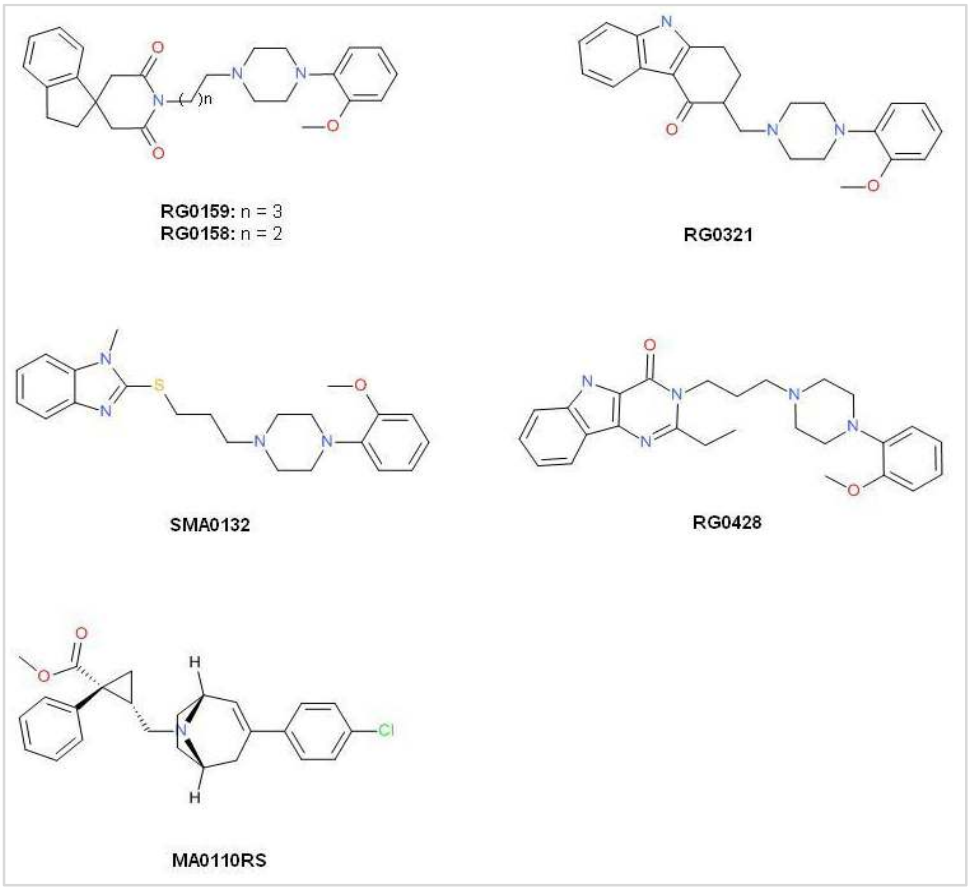


Fig. 2. Compounds under study that showed non-selective inhibition of Nav1.7 and Nav1.5

4. CONCLUSION

Overall pharmaceutical companies, might take advantage of academic collaboration for the identification of new hits, with the advantage of increased chances to reduce costs as well share own research goals. Indeed the adherence to the Lilly OIDD program [2] allowed us to evaluate our molecules through cheminformatics tools in the Lilly OIDD web platform [7], which identified molecules to be candidates for the in-vitro screening as well through phenotypic and target-based assays modules which represent interesting therapeutic areas [3].

In conclusion, a series of abandoned compounds have been rescued and repositioned for a new opportunity of research. In this perspective, the above-mentioned compounds will be subject of further investigations concerning the structural properties as inhibitory ligands of Nav1.7 as well as structural relationship in the selectivity between Nav1.7 and Nav1.5.

Nowadays and still more in the future, approaches based on re-use and re-evaluation will be a necessity in every context of pharmaceutical research. Therapeutic development is a costly ($2 billion or more), complex and time-consuming process up to about 14 years. The drug pipeline is negatively affected because of a 95 percent failure rate. Bringing together the newest technology from pharmaceutical companies with academic resources should become part of highly efficient and time-frames reduced workflows to new opportunities for better therapeutics [21,22]. It must be noted as accessing the OIDD program also allows for synthetic facilities [2,3].

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors thank the Eli Lilly & C. for allowing to participate in the Lilly Open Innovation Drug Discovery (OIDD) program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12115