BUKTI KOREPONDENSI

JURNAL ILMIAH

JURNAL INTERNASIONAL BEREPUTASI DAN BERFAKTOR DAMPAK WoS IF > 1,0 (SYARAT KHUSUS)

NO	JENIS ISIAN	ISIAN
1	Judul Artikel	Cis-2 and trans-2-eicosenoic fatty acids are novel inhibitors for Mycobacterium tuberculosis protein tyrosine phosphatase A
2	Penulis	 Lalu Rudyat Telly Savalas; 2. Baiq Repika Nurul Furqon; 3. Dina Asnawati; 4. Jannatin 'Ardhuha; 5. Prapti Sedijani; 6. Saprizal Hadisaputra; 7. Baiq Nila Sari Ningsih; 8. Jufrizal Syahri
3	Nama Jurnal	Acta Biochimica Polonica
4	Tahun Terbit	2020
5	Volume Jurnal	67
6	Nomor Jurnal (Opsional)	2
7	Halaman	219-223
8	ISSN	ISSN / eISSN 0001-527X / 1734-154X
9	Penerbit	The Polish Biochemical Society and the Polish Academy of Sciences
10	DOI	https://doi.org/10.18388/abp.2020_5201
11	Alamat Web Jurnal	https://ojs.ptbioch.edu.pl/index.php/abp/
12	URL Dokumen	https://ojs.ptbioch.edu.pl/index.php/abp/article/view/5201
13	Link Index	https://ojs.ptbioch.edu.pl/index.php/abp/about
14	Apakah ini syarat khusus	Ya (First author dan corresponding author, faktor dampak > 1,0)
15	Keterangan (Opsional)	Memenuhi syarat khusus faktor dampak > 1,0 (https://mjl.clarivate.com/journal-profile) (akun MJL institusional dibutuhkan untuk melihat informasi detil jurnal dimaksud menurut pengindeks Clarivate Analytics), atau https://ojs.ptbioch.edu.pl/index.php/abp/about, atau sumber lain: https://academic-accelerator.com/Impact-Factor-IF/Acta- Biochimica-Polonica

KRONOLOGI KOREPONDENSI

No	Tanggal	Aktivitas	Keterangan
1	10 Maret 2020	Submission acknowledgement	Email dari Editor Acta Biochimica
			Polonica (ABP)
2	10 Maret 2020	Permintaan melengkapi submission	Diskusi di OJS ABP
3	11 Maret 2020	Tambahan kelengkapan submsission	Via OJS ABP
4	11 Maret 2020	Tambahan kelengkapan submission	Email ke ABP
5	20 April 2020	Diskusi proses review	Saran reviewer potensial
6	5 Juni 2020	Acceptance dengan revisi minor	Diskusi di OJS, konfirmasi
			acceptance telah dikirim via email
			tetapi tidak masuk mailbox
			author. Acceptance dan revisi
			disampaikan via OJS.
7	5 Juni 2020	Tanggapan rerhadap permintaan revisi minor	Diskusi di OJS
8	5 Juni 2020	Cover letter revisi manuskrip	Dikirim author ke OJS ABP
9	6 Juni 2020	Full manuskrip setelah revisi	Dikirim author ke OJS ABP
10	8 Juni 2020	Copyedit	Dikirim author ke OJS ABP
11	14 Juni 2020	Permintaan proofreading author	Diskusi via email
12	15 Juni 2020	Jawaban author terhadap permintaan proofreading akhir	Via email

Dokumen di bawah ini disusun menurut kronologi seperti pada tabel di atas.

K1. Submission Acknowledgment

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Less		Biochimica Polonica. With the online journal management system that we are using, you will be able to track its progress through the editorial process		
		by logging in to the journal web site:		
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K2. Permintaan melengkapi metadata

[ABP] New Submission without Metadata completed 5201



Mrs. Malgorzata Goraj-Basaj (mgb)

Dr hab. Katarzyna Potrykus (kpotrykus)

Lalu Rudyat Telly Savalas (savalas123)

Messages

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Note	From
Lalu Rudyat Telly Savalas:	mgb
Dear Authors,	2020-03-10 11:23 PM
Thank you for the submission 5201 entitled: "Cis-2 and trans- 2-eisocenoic fatty acids are novel inhibitors for Mycobacterium tuberculosis Protein tyrosine phosphatase A," in Acta Biochimica Polonica.	
However, according to Instructions for Authors three names with affiliations and mailing addresses of potential reviewers should be added as comments.	
You are also asked to send the e-mails of ALL the Authors as according to the rules of COPE all Authors must be notified about the progress of the submission.	
Without this step manuscript could not be processed.	
Sincerely	
Małgorzata Basaj	
Mrs. Malgorzata Goraj-Basaj Polish Biochemical Society, Warsaw abp@ptbioch.edu.pl	

K3. Tambahan kelengkapan submission

	 Thank you for your response. I noted the instruction but missed the steps during submission in the new OJS system. Can I just add the requested potential reviewers as words file attached to this presubmission menu? Also, for the the email address of the co-authors, how can I edit and add them via the system? (I would suggest that the email addresses of co-authors denoted as compulsary during submission process, so that the same problem can be avoided in the future. Alhthouh I understand that it is stated in the guidelines). Thank you and I will be back to fullfil the request. Kind regards, LR Telly Savalas 	savalas123 2020-03-11 12:54 AM
	The e-mails please can be added at this step only by me so write them in the message. As to the reviewers you can attached them as word file also in the message not tin the submission menu. Sincerely Małgorzata Basaj	mgb 2020-03-11 09:02 AM
•	Thank you. It is noted. I just send you the requested information via email. I look forward for a positive outcome. Kind regards, Telly	savalas123 2020-03-11 02:09 PM
	 Recommended reviewers for submission 5201 Dessy Natalia, Institut Teknologi Bandung, Indonesia, dessy@chem.itb.ac.id Carlos Polanco, Universidad Nacional Autónoma de México, E-mail: polanco@unam.mx Magdalena Druszczyńska, University of Lodz, E-mail: majur@biol.uni.lodz.p 	mgb 2020-03-11 10:11 PM

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Chemist 378 Dn Wednesday, 11 March 2020, 4:05:58 pm GMT+8, abp@ptbioch.edu.pl> wrote: DAD 2005 850 Palad Product Halal Product You have a new notification from Acta Biochimica Polonica: HKI NTB There is new activity in the discussion titled "[ABP] New Submission "Cis-2 and trans-2-eisocenoic fatty acids are novel inhibitors for Mycobacterium tuberculosis Protein tyrosine phosphatase A". ishak alim Link: https://ojs.ptbioch.edu.pl/index.php/abp/authorDashboard (submission/5201) Kelas Laut Małgorzata Basaj Konsultasi Anak The following message is being delivered on behalf of Acta Biochimica Polonica. UNRAM-dikti Jownload all attachments as a zip file										
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K5. Diskusi proses review

[ABP] A message regarding Acta Biochimica Polonica 5201

Participants

Mrs. Malgorzata Goraj-Basaj (mgb)

Dr hab. Katarzyna Potrykus (kpotrykus)

Lalu Rudyat Telly Savalas (savalas123)

Messages

Ν	ote	From
	Dear Dr Savalas,	mgb
	We have trouble in finding a willing person for commenting your paper.	2020-04-20 10:01 PM
	Could you kindly send us three names with mailing addresses and affiliations?	
	It would speed up the reviewing process.	
	Sincerely	
	Małogrzata Basaj	
	The following message is being delivered on behalf of Acta Biochimica Polonica.	
•	Dear Ms Basaj, thank you for your email. Herewith I recommend	savalas123
		5474145725
	potential reviewers for my manuscript: 1. Dr. Roil Bilad,	2020-04-24
	potential reviewers for my manuscript: 1. Dr. Roil Bilad, Biochemical Process, Universiti Petronas Malaysia. Email:	
		2020-04-24
	Biochemical Process, Universiti Petronas Malaysia. Email:	2020-04-24
	Biochemical Process, Universiti Petronas Malaysia. Email: mroil.bilad@utp.edu.my 2. Prof. Iman Permana Maksum, Dept of	2020-04-24
	Biochemical Process, Universiti Petronas Malaysia. Email: mroil.bilad@utp.edu.my 2. Prof. Iman Permana Maksum, Dept of Chemisty, University Padjajaran Bandung Indonesia. Email:	2020-04-24
	Biochemical Process, Universiti Petronas Malaysia. Email: mroil.bilad@utp.edu.my 2. Prof. Iman Permana Maksum, Dept of Chemisty, University Padjajaran Bandung Indonesia. Email: ip_maksum@unpad.ac.id 3. Dr. Jaspreet Kaur Dhanjal, National	2020-04-24
	Biochemical Process, Universiti Petronas Malaysia. Email: mroil.bilad@utp.edu.my 2. Prof. Iman Permana Maksum, Dept of Chemisty, University Padjajaran Bandung Indonesia. Email: ip_maksum@unpad.ac.id 3. Dr. Jaspreet Kaur Dhanjal, National Institute of Advance Science, Japan, Email:	2020-04-24 05:47 AM
	Biochemical Process, Universiti Petronas Malaysia. Email: mroil.bilad@utp.edu.my 2. Prof. Iman Permana Maksum, Dept of Chemisty, University Padjajaran Bandung Indonesia. Email: ip_maksum@unpad.ac.id 3. Dr. Jaspreet Kaur Dhanjal, National Institute of Advance Science, Japan, Email: jaspreetk.dhanjal@aist.go.jp 4. Dr. Ni Nyoman Sri Budayanti,	2020-04-24 05:47 AM

K6. Acceptance dengan perbaikan minor

Dear Telly,

I've send the message with the decision to your email address through the system, but maybe it ended up in your spam folder (?). Anyway, I am attaching the message below, kpotrykus 2020-06-05 10:41 AM

Cheers,

Kasia

From: "Dr hab. Katarzyna Potrykus" <k.potrykus@abp.ptbioch.edu.pl>

To: "Lalu Rudyat Telly Savalas" <telly@uram.ac.id>, "Baiq Repika Nurul Furqon" <refika.nf007@gmail.com>, "Dina Asnawati" <dinaasnawati@gmail.com>, "Jannatin 'Ardhuha" <j.ardhuha@unram.ac.id>, "Prapti Sedijani" <praptisedijani@unram.ac.id>, "Saprizal Hadisaputra" <rizal@unram.ac.id>, "Baiq Nila Sari Ningsih" <6110220044@email.psu.ac.th>, "Jufrizal Syahri" <jsyachri@umri.ac.id>

Subject: [ABP] Editor Decision reference number 5201

reference number 5201 entitled: "Cis-2 and trans-2-eisocenoic fatty acids are novel inhibitors for Mycobacterium tuberculosis Protein tyrosine phosphatase A"

Dr Lalu Rudyat Telly Savalas, Baiq Repika Nurul Furqon, Dina Asnawati, Jannatin 'Ardhuha, Prapti Sedijani, Saprizal Hadisaputra, Baiq Nila Sari Ningsih, Jufrizal Syahri

Dear Dr Lalu Rudyat Telly Savalas, Baiq Repika Nurul Furqon, Dina Asnawati, Jannatin 'Ardhuha, Prapti Sedijani, Saprizal Hadisaputra, Baiq Nila Sari Ningsih, Jufrizal Syahri

I am happy to inform you that your paper can be accepted for publication in Acta Biochimica Polonica after minor revisions.

Please find the comments below this letter.

Please consider carefully the report and amend the manuscript accordingly. You are also kindly requested to answer in detail to all comments and describe them in the accompanying letter which should be attached as the first page of the revised manuscript.

When preparing the revised version of your manuscript please refer to the Instructions to Authors and to the latest issue of Acta Biochimica Polonica available on-line at https://ojs.ptbioch.edu.pl/index.php/abp/about/submissions to follow the style and requirements of the journal.

It is assumed that the final manuscript will be accepted by all the authors.

Please send the revised version of the article (with changes marked in color) and the cover letter describing the changes made to the manuscript by the system (under Revisions).

Thank you in advance for your prompt revision, Sincerely

Dr hab. Katarzyna Potrykus University of Gdańsk, Gdańsk, Poland k.potrykus@abp.ptbioch.edu.pl

Reviewer D:

The new idea of verification of potential inhibitors of protein tyrosine phosphatase (PtpA) of *Mycobacterium tuberculosis* is sound and reasonable. The authors extended this attracting and straightforward approach to test the potential inhibitors such as *cis*-2 and *trans*-2 eicosenoic fatty acids. The calculated IC₅₀ values as 11.26, 8.20 and 27.97 mM for *trans*-2, *cis*-2 and *trans*-11-eicosenoic fatty acid, clearly indicates it potential. Additionally, docking analysis of PtpA inhibition by *cis*-2-eicosenoic acid is identyfying four aminoacids Asn₁₄, Ile₁₅, Cys₁₆, Arg₁₇ as binding with strong interaction with cDocker energy -37,1939 kcal/mol. This constitutes the goals and novelty of the manuscript.

On the other minor note the following suggestion/correction should be incorporated into the text before publication:

- 1. 1. Page 5, line 15 the word "was" should be replaced with "is"
- Page 6. line 18 the words "In agreement with inhibition assay" should be moved and incorporated at the end of the sentence.
- Page 8. line 14. The sentence should be corrected by insertion "that" before "rather" and by inserting at the end of the sentence the words "strongly interact with PtpA"

The manuscript is of <u>good quality</u> and importance and <u>is written</u> in order to meet the standard for the articles published in *Acta Polonica Biochimica*. I certainly recommend it for publication in the current version with <u>incorporation of these small changes</u>.

Recommendation: Revisions Required

The following message is being delivered on behalf of Acta Biochimica Polonica.

Dear	Dr.	Potrykus,	
------	-----	-----------	--

thank you for you notification. I have attached the requested revision as well as a cover letter for the revision.

savalas123 2020-06-05 02:11 PM

I hope that the raised issues are solved.

Should there is furhter concern, please do not hesitate to contact me.

Kind regards,

Telly

P.S. It seems that I got trouble in receiving the requested revision. I cannot find it in spam box as well, but thank you that this OJS platform works well.

Add Message

Х

K7. Respons terhadap permintaan revisi minor

Copyediting file submission 5201



Mrs. Malgorzata Goraj-Basaj (mgb)

Dr hab. Katarzyna Potrykus (kpotrykus)

Lalu Rudyat Telly Savalas (savalas123)

Messages

Note	From
Please find our Words file for submission 5201. As requested, we have omitted markup to allow further process.	savalas123 2020-06-05 03:34 PM
Thank you.	
Kind regards,	
Dr. Lalu Rudyat Telly Savalas	
savalas123, Novel Mtb PtpA Inhibitor submitted to Act Biochim Polonica LRT Savalas submission 5201 Copyediting.docx	

Add Message

K8. Cover letter revisi manuskrip

Dear ABP Editor,

Thank you for the requested revision for submission 5201. In respoding to the requested revision, herewith I list the revision we made:

1. Issue 1: Page 5, line 15 the word "was" should be replaced with "is"

Original text: "Lysate of untransformed *E. coli* BL21was used as control in each experiments."

Replaced with: "Lysate of untransformed *E. coli* BL21 is used as control in each experiments."

2. Issue 2: Page 6. line 18 the words "In agreement with inhibition assay" should be moved and incorporated at the end of the sentence.

Original text:

"In agreement with inhibition assay, docking of PtpA with *trans*-11-eicosenoic acid prone to have weaker interaction in term of both energy and the distance of amino acid residues."

Replaced with:

"Docking of PtpA with *trans*-11-eicosenoic acid prone to have weaker interaction in terms of both energy and the distance of amino acid residues which is in agreement with the inhibition assay."

3. Issue 3: Page 8. line 14. The sentence should be corrected by insertion "that" before "rather" and by inserting at the end of the sentence the words "strongly interact with PtpA"

Original text:

"It is somehow surprising that a small molecule such as eicosenoic acid can strongly interact with PtpA, as many researchers have reported rather complex inhibitors."

Replaced with:

"It is somehow surprising that a small molecule such as eicosenoic acid that rather complex inhibitors as many researchers have reported strongly interacts with PtpA."

To conclude this letter, I have the opinion that the requested revision has been properly made. I also confirm that the revised manuscript refers to the latest Author Guidelines. Thank you and I look forward to hearing from you.

Kind regards, Dr. Lalu Rudyat Telly Savalas Dept of Chemistty, Faculty of Teacher Training and Education University of Mataram Jl. Majapahit No. 62 Mataram Nusa Tenggara Barat, Indonesia 83125 Email <u>telly@unram.ac.id</u>; <u>rudyat_telly@yahoo.com</u>

K9. Manuskrip yang telah direvisi

Cis-2 and trans-2-eicosenoic fatty acids are novel inhibitors for

Mycobacterium tuberculosis protein tyrosine phosphatase A

Lalu Rudyat Telly Savalas ^{1*}, Baiq Repika Nurul Furqan², Dina Asnawati², Jannatin 'Ardhuha³, Prapti Sedijani⁴ Saprizal Hadisaputra¹, Baiq Nila Sari Ningsih^{1,5}, Jufrizal Syahri⁶

- ¹ Department of Chemistry Education, Faculty of Teacher Training and Education, University of Mataram, Indonesia; telly@unram.ac.id
- ² Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram;
- ³ Department of Physics Education, Faculty of Teacher Training and Education, University of Mataram;
- ⁴ Department of Biology Education, Faculty of Teacher Training and Education, University of Mataram;
- ⁵ Department of Chemistry, Faculty of Science, Prince of Songkhla University, Thailand
- ⁶ Department of Chemistry, Universitas Muhammadiyah Riau, Indonesia

* Correspondence: telly@unram.ac.id; Tel.: (+62 (0370) 623873)

Abstract: Small protein tyrosine phosphatase (PtpA) of *Mycobacterium tuberculosis* (Mtb) is attributed to the development of latent tuberculosis infections, and hence becomes an interesting target for drug development. In this communication, inhibition of PtpA by naturally occurring fatty acids: *cis*-2 and *trans*-2-eicosenoic acids is investigated. Mtb PtpA was heterologously expressed in *Escherichia coli*, and the activity of PtpA was inhibited by *cis*-2 and *trans*-2 eicosenoic fatty acids. Both compounds showed strong inhibition of PtpA activity, with IC₅₀ at low micromolar concentrations. In comparison, *trans*-11-eicosenoic acid only slightly inhibited PtpA. *In silico* analysis confirmed the inhibition of PtpBA by *cis*-2-eicosenoic acid by formation of several hydrogen bonds. These findings show that *cis*-2 and *trans*-2 eicosenoic fatty acids are potential candidates for latent tuberculosis inhibitors.

Keywords: *Mycobacterium tuberculosis*, latent infection, Pprotein tyrosine phosphatase A, *cis*-2 and *trans*-2 eicosenoic fatty acids

1. Introduction

Mycobacterium tuberculosis pathogen is one of the most devastating pathogens with high mortality all over the world. The death toll caused by this infection is worsened by increasing HIV infection cases. This figure is even more complicated by the development of antibiotic resistant strains: multi-drug resistant tuberculosis (MDR TB) and extensively drugresistant TB (XDR TB). The battle against TB has an additional front, i.e. latent TB infection (LTBI). It is estimated that 1.7 billions of people, or approximately one quarter of the world's population, are infected with these bacteria in the latent infection fashion (World Health Organization, 2019; Houben & Dodd, 2016), and 10% of those individuals develop active infection at a later stage of their life (Vynnycky & Fine, 2000; Stutz *et al.*, 2018).

The ability of the bacteria to avoid the acidic lysosomal degradation within host macrophages has been recognized as one of the survival mechanisms of *M. tuberculosis* from phagolysosome degradation, and leads the bacterium to its latent phase (Pieters & Gatfield, 2002), where it further utilizes nutrition from its host for long term dormancy (Mali & Meena, 2018). Although several bacterial survival factors, such as the protein tyrosine phosphatases, bacterial lipoarabinomannan (LAM) and protein kinase G (PknG) have been extensively suggested to be involved in the latent infection (Li & Xie, 2011; Janssen *et al.*, 2012), the mechanism by which latent TB infection develops into its active state is not fully understood. Nevertheless, inhibition of latency factors opens up the research field to combat bacteria even before latency is established.

Among the proteins associated with the development of latent TB infection is protein tyrosine phosphatase A (PtpA). This protein is known to be responsible for inhibition of fusion even between phagosome and lysosome. In a normal endocytosis pathway, once the bacterium is engulfed by the macrophage cell, the resulted mycobacterial-laden phagosome recruits host vacuolar- H⁺-ATPase (V-ATPase) that acidifies the endosome, a precondition required later when it fuses with the lysosome and thus ensuring that a suitable environment for hydrolytic enzymes of the lysosome is formed (Sun-Wada *et al.*, 2009; Stutz *et al.*, 2018; Upadhyay *et al.*, 2018). However, bacterium develops a survival strategy by secreting PtpA that permeates the phagosome membrane and binds to the subunit H of V-ATPase in the macrophage cytosol. PtpA is also reported to dephosphorylate vacuolar sorting protein VPS33B which is required for the fusion of endocytic organelles. The binding of subunit H of V-ATPase and dephosphorylation of VPS33B by mycobacterial PptA are concerted evens that account for the hinderance of bacteria to enter phagolysosome degradation (Bach *et al.*, 2008; Wong *et al.*, 2011; Korb *et al.*, 2016). PtpA was also reported to supress the host innate immunity by regulating host gene expression (Wang *et al.*, 2017). From this point of view, PtpA, along with other effector proteins, becomes an interesting target for anti-latent TB drug development.

Several attempts to discover novel drugs benefitted from the progress in the computer aided drug discovery (CAD). A recent example of this approach has been underlined by Zhang and co-workers who reported a thiobarbiturate compound as a novel Mtb PtpB inhibitor with an IC₅₀ of 22.4 μ M (Zhang *et al.*, 2019). Furthermore, a thiosemicarbazone compound predicted by molecular modelling has been synthesized and revealed its inhibitory effect on Mtb PtpA with a low micromolar IC₅₀ (Sens *et al.*, 2018). A comprehesive review of *in silico* studies targetting tubercular protein is presented by de Oliveira Viana *et al.* (de Oliveira Viana *et al.*, 2018). A recent *in silico* study by Dhanjal and co-workers has suggested that eicosenoic fatty acid derivative, the *trans*-2 eicosenoic acid. might inhibit Mtb PtpA and a related phosphatase of Mtb, PtpB (Dhanjal *et al.*, 2014). The present study aims at testing the ability of *trans*-2-eicosenoic fatty acid to inhibit Mtb PtpA.

2. Materials and Methods

2.1 Materials

Plasmid and Escherichia coli strains

Recombinant plasmid pET30b-PtpA was a kind gift from Prof. Yossef Av-Gay, Univesity of British Columbia, Canada. All-The plasmids wasere maintained in *Escherichia coli* XL1-Blue, and for expression, *E. coli* BL21(DE3) was used. Bacteria were grown in LB medium containing 0.5% yeast extract, 1% NaCl, 1% bacto trypton and 30 μ g/mL kanamycin (USP Biobasic). Agar LB medium was made by addition of 2% bacto agar. All ingredients were from major biochemical vendors.

Chemicals

All reagents for buffers were from major chemical vendors. *Cis*-2-eicosenoic and *trans*-2-eicosenoic fatty acids were purchased from Larodan AB (Sweden). *Trans*-11-eicosenoic fatty acid and *para*-nitrophenyl phosphate were from Sigma-Aldrich.

2.2. Methods

2.2.1 Expression of Mtb protein tyrosine phosphatase A in E. coli

Recombinant plasmid pET30b-PptpA was introduced into competent *E. coli* BL21(DE3) cells by electroporation (Gene Pulser Electroporation Systems, Bio-Rad). For PtpA production, an overnight pre-culture of transformed cells was added to 250 mL LB medium in an Erlenmeyer flask containing kanamycin. Cells bearing PtpA gene at log phase (OD₆₀₀ of c.a. 0.6) were induced by addition of 0.5 mM isoprophyl beta-thiogalactose (IPTG, Sigma-Aldrich) and kept in a rotary shaker for 4 hours at 37°C, 250 rpm. Cells were harvested by centrifugation at 3500 rpm and cell pellet was resuspended in phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄ and 2 mM KH₂PO₄) prior to lysis by 10

cycles of sonication (30 seconds sonication and 30 seconds pause). Clear lysate containing PtpA was obtained by centrifugation at 12,000 rpm, 4°C for 30 min.

Protein concentration was determined by bicinchoninic acid (BCA kit, Thermo scientific). Sodium dodecylsufate polyacrylamide gel electrophoresis was performed to show the expression of PtpA in *E. coli*. Samples were subjected to electrophoresis in 12.5% polyacrylamide gel.

The activity PtpA was tested for its phosphatase activity against *para*-nitrophenyl phosphate (p-NPP) as modified from (Mascarello et al., 2013). Reaction mixture contained 6 mM pNPP substrate and 100 μ M imidazole was added to final volume of 180 μ L, followed by preincubation at 37°C for 5 minutes prior to addition of 20 μ L of PtpA (1 μ g/ μ L). Reaction was allowed to proceed and the resulted *para*-nitrophenol was measured at 410 nm every 2 minutes for 30 minutes.

2.2.2 Inhibition of PtpA by eicosenoic fatty acids

To test the inhibitory effect of eicosenoic fatty acids, PtpA was allowed to hydrolyze substrate *para*-nitrophenyl phosphate in the absense or presense on various concentrations of *trans*-2-eicosenoic acid, *cis*-2-eicosenoic acid, and *trans*-11-eicosenoic acid, ranging from 0 μ M to 30 μ M, for 30 minutes at 37°C. Lysate of untransformed *E. coli* BL21 was used as control in each experiments.

The inhibitory effect of eicosenoic fatty acid isomers on the activity of PtpA was depicted as the decrease in absorbance (A_{410} nm) by the increment concentration of eicosenoic fatty acids. The IC₅₀ values for each eicosenoic fatty acid were calculated with the use of Prism 7 (GraphPad).

2.2.3 Docking

Interaction of eicosenoic fatty acids with PtpA that lead to inhibitory of phosphatase activity was analyzed with the Discovery studio (Accelrys, San Diego, CA, USA). The PtpA protein that contains native ligand glycerol (PDB accession number 1U2Q) was used for docking with each of the eicosenoic fatty acids. Ligands, i.e. the eicosenoic fatty acids, were prepared by Chemdraw (Fig. 1B).

3. Results

3.1 Expression of PtpA in E. coli

PtpA₇₂ cloned into the pET30b plasmid, was successfully expressed in *E. coli* BL21(DE3) under T7 promoter and induced by 0.5 mM isopropyl beta-thiogalactose (IPTG) as described by Studier *et al* (Studier et al., 1990). PtpA appears as a protein of c.a. 18 kDa (Figure 1A), which is in agreement with another report (Chiaradia et al., 2008). Activity of overexpressed PtpA was assayed by measuring its ability to hydrolyze *para*-nitrophenyl phosphate (pNPP). The released *para*-nitrophenol gave a typical yellow colour of reaction mixture which is measured spectrophotometrically at 410 nm (Chiaradia et al., 2008).

3.2. Inhibition of PtpA with eicosenoic fatty acids

Inhibition of PtpA by *trans*-2 and *cis*-2-eicosenoic acids is shown in Figure 2. It is shown that the *trans*-2 eicosenoic acid, and to a higher extent the *cis*-2-eicosenoic acid, are capable of inhibiting PtpA.

3.3. Docking analysis

Interaction study of PtpA with the *cis*-2-eicosenoic acid is depicted in Fig. 3. The fatty acid shows interaction with four amino acid residues of PtpA, i.e. Asn₁₄, Ile₁₅, Cys₁₆ and Arg₁₇ with binding energy of -37.1939 kcal/mol. Strong interaction of PtpA is also found

with *trans*-2-eicosenoic acid via two potential amino acid residues, i.e. Thr_{12} , Gly_{14} , and to a lesser degree with Arg_{17} . <u>On the other hand</u>, <u>D</u>docking of PtpA with *trans*-11-eicosenoic acid reveals it to be prone to have weaker interaction in terms of both, the energy and the distance of amino acid residues, which is in agreement with the inhibition assay results. Binding parameters of the tested eicosenoic fatty acids are summarized in Table 1.

	cDocker energy	Binding interaction
Compound	(kcal/mol)	(amino acid residue)
cis-2-eicosenoic acid	-37,1939	Asn14, Ile15, Cys16, Arg17
trans-2-eicosenoic acid	-33,0076	Thr ₁₂ , Gly ₁₃ , Arg ₁₇
trans-11-eicosenoic acid	-28,3423	Gly ₁₃ , His ₄₉

Table 1. Energy and binding interaction of co-crystalized Mtb PtpA (1U2Q)

with eicosenoic fatty acids

4. Discussion

The role of phosphatases in the progress of infection has been of interests of many researchers, since these proteins are identified in various bacterial pathogens, such as *Listeria monocytogenes, Mycobacterium tuberculosis, Pseudomonas aeruginosa, Streptococcus agalactiae, Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhimurium, etc.* (Sajid et al., 2015). The study areas could include identification of their host interacting partners and be followed by elucidation of the downstream interferences they cause. The next immediate interest, unsuprisingly, is to find a way to inhibit the secreted bacterial phosphatases, and thus prevent their involvement in the development of infections and diseases.

As TB remains a global concern that belongs to one of the main targets in the Sustainbable Development Goal (SGD) in health, prevention of latent TB turns to be one of the focal points, in addition to other TB eradication efforts, such as development of new vaccines, novel drugs, as well as improvement of diagnosis. Our current knowledge on roles of mycobacterial phosphatase effectors that are secreted by Mtb into its host's cells_enroute its degradation pathway has led researchers to explore inhibitors of Mtb phosphatases. The endeavour to seek new potential inhibitors for Mtb virulence proteins and drugs againts TB is nowadays approached by differents strategies. Among those strategies are direct screening of natural compounds, *in silico* screening of phosphatase inhibitors, and synthesis of novel compounds or modified compounds predicted to be able to inhibit Mtb phosphatases.

The study presented here employs an *in silico* report that has underlined the potential inhibitory effect of *trans*-2-eicosenoic fatty acid on Mtb PtpA (Dhanjal et al., 2014). Our data confirmed for the first time that *trans*-2-eicosenoic fatty acid strongly inhibits PtpA, with an IC₅₀ of 11.26 μ M. Interestingly, its *cis* isomer (*cis*-2-eicosenoic fatty acid) showed an even stronger inhibition to PtpA, with an IC₅₀ of 8.20 μ M. The ability of these eicosenoic acids to inhibit PtpA is comparable to the other PtpA potential inhibitors, such as chalcone derivatives (Mascarello et al., 2010) and a patented compound (Organization, 2012). In contrast, the *trans*-11-eicosenoic acid isomer of those compounds showed a much higher IC₅₀ value, i.e. 27.97 μ M and only slightly inhibited PtpA (Fig. 2). The fact that both *trans*-2 and *cis*-2 eicosenoic acids strongly inhibit PtpA, whereas *trans*-11 does not, shows that the double bond position contributes more to the inhibitory effect of the eicosenoic acids than its *cis* or *trans* stereochemistry (Figure 2). It is somehow surprising that a small molecule such as eicosenoic acid, rather than complex inhibitors as many researchers have reported, strongly interacts with PtpA.

5. Conclusions

These findings show that both, the *cis*-2 and *trans*-2-eicosenoic fatty acids are potential candidates for PtpA inhibitors. A further study is deemed necessary in order to investigate whether these compounds bind specifically to the Mtb's phosphatase by comparing their inhibitory effects on human phosphatases. Additionally, it is also important to employ an assay to prove whether these compounds are capable of preventing Mtb latent infection *in vivo*.

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Conflicts of Interest: All authors declare no conflict of interest.

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Figure Legends

Figure 1

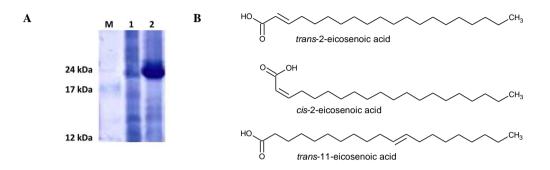
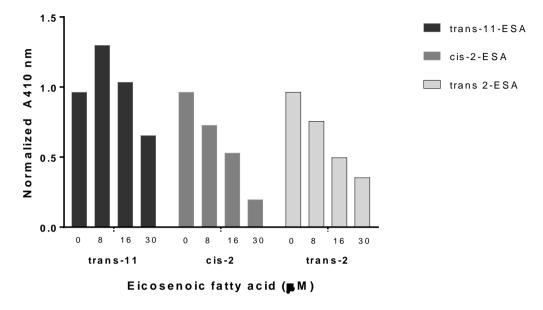


Figure 1. A. In each lane, 20 μg of samples were loaded onto 4.5% focusing gel and 12.5% resolving polyacrylamide gel for separation. Overexpressed PtpA in *Escherichia coli* BL21(DE3) appears as a major band at 18 kDa which corresponds to PtpA (lane 2). Lane 1 is uninduced sample. B. Three isomers of eicosenoic fatty acid structures generated by Chemdraw, tested for their inhibitory effect on Mtb PtpA activity: *trans*-2-eicosenoic acid, *cis*-2-eicosenoic acid and *trans*-11-eicosenoic acid.

Figure 2



Inhibition of PtpA by variuos concentration of eicosenoic fatty acids

Figure 2. Inhibition study of overexpressed PtpA with increasing concentration of eicosenoic fatty acids (ESA) at 0, 8, 16 and 30 μ M. The *trans*-2-eicosenoic and *cis*-2-eicosenoic strongly inhibit PtpA, whereas *trans*-11-eicosenoic acid only slightly inhibits PtpA. All measurements were performed twice and data were normalized to the control *E. coli* lysate. IC₅₀ values of PtpA inhibition by *trans*-2, *cis*-2 and *trans*-11 eicosenoic acid were calculated with the Prism 7 software (GraphPad) and it was revealed that the IC₅₀ values were 11.26, 8.20 and 27.97 μ M for *trans*-2, *cis*-2 and *trans*-11-eicosenoic fatty acid, respectively. ESA: Eicosenoic Fatty Acid.



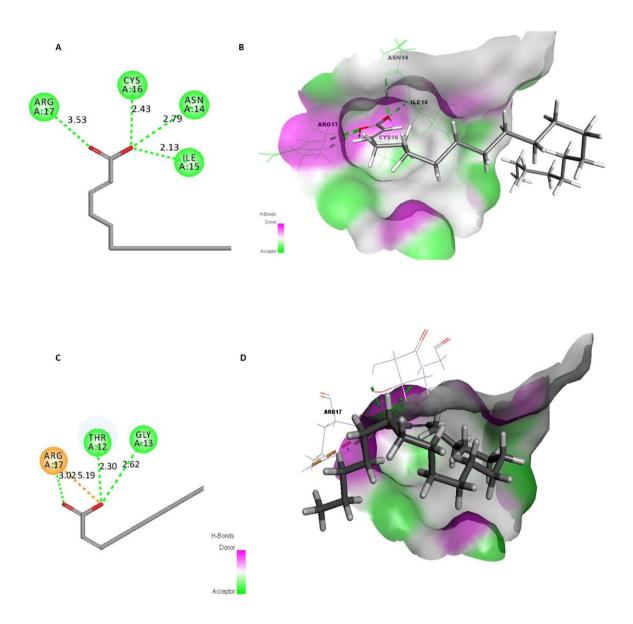


Figure 3. A and B: Docking analysis of PtpA inhibition by *cis*-2-eicosenoic acid. Four amino acid residues of PtpA are in close proximity to the *cis*-2-eicosenoic acid. C and D: Docking analysis of PtpA inhibition with *trans*-2-eicosenoic acid. Three amino acids are in close proximity to *trans*-2-eicosenoic acid.

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