Upsala Journal of Medical Sciences

ISSN: 0300-9734 (Print) 2000-1967 (Online) Journal homepage: informahealthcare.com/journals/iups20

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To cite this article: Anna Zorzet (2014) Overcoming scientific and structural bottlenecks in antibacterial discovery and development, Upsala Journal of Medical Sciences, 119:2, 170-175, DOI: 10.3109/03009734.2014.897277

To link to this article: https://doi.org/10.3109/03009734.2014.897277

Upsala Journal of Medical Sciences. 2014; 119: 170–175

informa
healthcare

REVIEW ARTICLE

Overcoming scientific and structural bottlenecks in antibacterial discovery and development

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Abstract

Antibiotic resistance is becoming an increasing threat, with too few novel antibiotics coming to market to replace those lost due to resistance development. Efforts by the pharmaceutical industry to screen for and design novel antibacterials have not been successful, with several companies minimizing or closing down their antibacterial research units, leading to a loss of skills and know-how. At the same time, antibiotic innovation in academia is not filling the void due to misaligned incentive structures and lack of vital knowledge of drug discovery. The scientific and structural difficulties in discovering new antibiotics have only begun to be appreciated in the latest years. Part of the problem has been a paradigm shift within both industry and academia to focus on 'rational' drug development with an emphasis on single targets and high-throughput screening of large chemical libraries, which may not be suited to target bacteria. The very particular aspects of 'targeting an organism inside another organism' have not been given enough attention. In this paper, researcher interviews have complemented literature studies to delve deeper into the specifics of the different scientific and structural barriers, and some potential solutions are offered.

Key words: *Academic drug discovery, antibiotic innovation, antibiotic resistance, novel antibiotics*

Introduction

While the problem of antibacterial resistance is becoming increasingly worse (1), the number of new antibiotics coming onto the market is dwindling (2,3). In the last 40 years, only two novel classes of antibiotics have been marketed, and those two classes were discovered before 1987 (4). In recent years, many attempts have been made to explain this lack of innovation, with many papers written on the difficulties in antibacterial discovery and development. Most of these papers focus on one of three identified components or bottlenecks: regulatory (5), financial (6), or scientific (4,7). Calls have also been made to create new economic models that will overcome these bottlenecks and to ensure that any novel antibiotic is used responsibly and with equitable access for all (8-12). This paper focuses on the structural barriers encountered by scientists involved in antibiotic discovery, reasons for the lack of innovation in the field of antibacterials in the pharmaceutical sector, and attempts to offer some possible solutions. Problems and potential solutions were identified through literature review, interviews with researchers in academia and the pharmaceutical industry, as well as participation in meetings on the subject.

When discussing discovery of novel antibiotics, it is important to note that there are different definitions of the word 'novel'. It is most often defined as an antibiotic that acts on a previously unexploited bacterial target. However, novel antibiotics could also hit unexplored areas of already used targets such as the protein translation machinery or the cell wall synthesis. Macrolides and chloramphenicol are for example still considered completely different classes, even though they both target the peptidyl transfer step of translation. Furthermore, antibiotics that are not strictly novel classes can still be extremely useful if they for example overcome a particular resistance problem. Therefore, it is important to

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(Received 20 January 2014; accepted 19 February 2014)

ISSN 0300-9734 print/ISSN 2000-1967 online
DOI: 10.3109/03009734.2014.897277

discuss what kinds of 'novel' antibiotics are most needed. Since drug development from discovery to finished product most often takes place over a decade or more, how do we ensure that we get the antibiotics we need, when we need them? There seems to be almost unanimous agreement that the biggest threat right now is multi-drug-resistant Gram-negative bacteria—but which Gram-negative bacteria? Gonorrhoea, with 106 million new cases each year (13), and where we have very few treatment options, multi-resistant Pseudomonas, or carbapenem-resistant enterobacteriaceae? For the latter group, a new broad-spectrum antibiotic would most certainly be life-saving, but is it feasible to try for a new 'panacea' antibiotic, or do we have to be content with targeting sub-groups of these bacteria? For gonorrhoea, it may actually be possible to use existing drugs that have not yet been approved for use for this indication, but someone then needs to pay for the necessary clinical trials.[1] The point here is: who is making these priorities? At present, it is still the pharmaceutical companies, looking at potential markets, but if we want to ensure that products meet current and future health needs, one factor that needs to be urgently addressed is how to form a mechanism for dealing with these prioritization questions. Such an analysis of the need for new compounds has to be continuous, must build on the global trends of antibiotic resistance and health burden, and should be performed by the public sector.

Innovation crisis in antibiotic discovery and development

In the last few years, several processes have been initiated trying to address the dearth of novel antibiotics. The Swedish EU presidency's focus on the need for innovative incentives to develop new antibiotics helped to raise the issue high on the agenda in the EU (14,15). Following the subsequent release of the EU Action plan on Antimicrobial resistance (AMR) (16) was the creation of a new programme entitled 'New Drugs for Bad Bugs' (ND4BB) within the Innovative Medicines Initiative (a public–private partnership, PPP). Another example of a PPP initiative on this topic is the collaboration between the Singaporean Agency for Science, Technology and Research and AstraZeneca to generate antibiotics for Gram-negative infections.[2] Examples from other fields include the Drugs for Neglected Diseases Initiative (DNDi) working with the Drug Discovery Unit at Dundee,[3] and the Medicines for Malaria Venture collaborating with Genzyme Corporation and the Broad Institute.[4] Other recent initiatives are the Joint Programming on AMR (JPIAMR),[5] where one of the priority topics in the strategic research agenda is the development of novel antibiotics and alternatives to antibiotics, and the GAIN act in the US. Among other things, the GAIN act uses extended market exclusivity as a carrot for pharmaceutical companies to create new antibiotics.[6] However many, also within industry, have questioned the usefulness of this approach in stimulating research and development (17), and there are also important implications for access in low-income countries that need to be considered.

The crisis in innovation is not exclusive to antibiotics, and many have pointed to a general need to revive innovative potential in the pharmaceutical industry (17). The shift in drug discovery approaches from a physiology-based to a target-based approach may be one cause. Focusing on processes rather than diseases in order to streamline and optimize drug discovery may have meant that proper scientific analysis suffered (18).

Antibiotic innovation is a particularly dire case and there have been huge losses in competence as the big pharmaceutical companies have gradually cut back on or completely shut down their research units on antibacterial substances. Much of the research in the field is now done in academia, or in smaller biotech companies that develop a molecule up to a point and then try to sell it to a big company that has the capacity and resources to conduct large clinical trials. Crossing the so-called 'valley of death', i.e. validating the commercial potential of a promising molecule is indeed challenging. Some of these challenges are explored in the section below ('Challenges in academic drug discovery').

Another factor that may be important in the decline of innovative new antibiotics is that the structure of the pharmaceutical industry has changed significantly since the 1950–60s. Nowadays, a molecule may take a very complex path through mergers and acquisitions from discovery through the different development stages. As an example, consider Ramoplanin™

[1]http://www.niaid.nih.gov/news/newsreleases/2013/Pages/GonorrheaTrial.aspx

[2]http://www.a-star.edu.sg/Media/News/Press-Releases/ID/1904/New-Deal-Aims-To-Reverse-Global-Rising-Tide-Of-Antibiotic-Resistance.aspx

[3]http://www.dundee.ac.uk/pressreleases/2009/prjune09/candidates.htm

[4]http://www.mmv.org/newsroom/press-releases/genzyme-corporation-and-mmv-announce-new-collaboration-advinus-therapeutics-

[5]http://www.jpiamr.eu

[6]http://www.gpo.gov/fdsys/pkg/BILLS-112s3187enr/pdf/BILLS-112s3187enr.pdf

(formerly known as A-16686), which is a glycolipodepsipeptide derived from bacteria of the genus Actinoplanes. Ramoplanin is active against vancomycin-resistant *Enterococcus* spp. (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-intermediate-resistant *Clostridium difficile* and was patented in 1980 in Italy. Since then, the molecule has been through numerous licencing events, mergers, and acquisitions as well as bankruptcies (see Box 1). One could argue that if the molecule was interesting enough, it would have become a drug, but do we really know this? At the very least, it would be worthwhile to explore the role of mergers and acquisitions for a selected number of potential antibiotic compounds.

Researchers in antibiotic discovery also need to start learning from their own and others' mistakes more systematically. Many interesting compounds have 'disappeared' for sometimes unknown reasons, before they ever came to phase II/III clinical trials. Were these compounds disregarded because they were truly not useful, or because of other considerations such as financial, which may not be valid any more, or toxicity issues that might be solvable decades later? A good place to start looking could be old pipeline analyses, patent literature, or material published by scientists previously working in the industry (19). If big pharmaceutical companies were also involved, they could contribute this information. One opportunity for such an analysis is the ND4BB programme mentioned above, where it is declared that 'all consortia participating in projects running under the ND4BB research programme will be expected to deposit data in the ND4BB data hub and work together to share data and experience as widely as possible amongst all programme members and the antibiotic community as a whole' (20).

Challenges in academic drug discovery

There seems to be a common notion among both the general public and policy-makers that if a lot of money is put into research this will in itself be enough to lead to new medicines. However, in reviewing literature, participating in meetings, and interviewing researchers in several different countries, it seems obvious that this conclusion is false and that much more than research is needed. One reason is that many (maybe even most) academic researchers are not primarily interested in the process of shepherding a molecule through the drug development process. University researcher incentives are almost exclusively based on publishing, and, after the initial discovery of a potential new molecule is published, establishing proof of concept that a molecule could be useful as a medicine is not deemed cutting-edge research. Collaborating with companies, patenting, etc. is also usually very counter-productive for publishing because of constraints on which knowledge can enter the public domain. Other issues with antibiotic discovery in the academic sector often include lack of knowledge of medicinal chemistry and the steps of developing a molecule into a drug after initial discovery. It may be worthwhile to develop a 'manual' or check-list of essential assays aimed at academic antibiotic drug discovery and development, as this expertise will otherwise have to be bought from consultants. Also, both medicinal chemistry and other expertise are essential to ensure a collaborative, productive environment of scientists from several different disciplines. In addition, if academia ventures further into drug development, skilled managers, not only skilled researchers, are needed, which may be hard to find unless very high salaries can be afforded (21).

The few researchers that do indeed wish to progress their molecule far enough so it may be sold to a biotech company will find that funding for this endeavour is very hard to come by. It is not pure research, so those sources of funding are usually not available. Venture capitalists are less prone these days to 'venture' and prefer much more proof before they will consider investing.

In Box 2, scientific areas have been listed, where increased knowledge would facilitate the discovery of new antibiotics, several of which have been suggested before (4,22,23) but have been gathered here for easy overview. It can be argued that breakthroughs cannot only be achieved by directing science, so funding of more explorative research should not be reduced. Instead increased funding for both targeted and explorative basic science is needed.

Alternatives to antibiotics

When discovery and development of new antibiotics are discussed, invariably alternatives to conventional antibiotics are brought up. Among the most common are antimicrobial peptides (AMPs) and bacteriophages, both of which have been mentioned as alternatives to antibiotics for decades. While these alternatives are certainly worth exploring, it may be good to note some of the reasons they will not be replacing conventional antibiotics, at least not any time soon. In the case of AMPs, they can have a direct antibacterial effect, but usually only under specific conditions (e.g. pH, ionic strength) and at quite high concentrations. To date it has been problematic to use their antimicrobial properties to develop them into pharmaceuticals. Instead, recent research focuses on their ability to recruit and

modulate the innate immune system (24). While this is an attractive approach, AMPs are fraught with problems like high production costs, toxicity, and susceptibility to proteases. It is very hard to achieve a high enough concentration in the body at the right site, making them unsuitable at least as replacement for systemic treatment. In the short term, AMPs are therefore best suited for topical and possibly inhalation applications. AMPs could also be used as complements to conventional antibiotics. The antibiotic clears the bacteria, and the AMP could reduce the inflammatory response, which would be very useful in e.g. sepsis. Certainly, AMPs are worth investigating further, and may in the long-term perspective become useful tools to combat bacterial infection. However, they are currently a complement to small-molecule antibiotics, not a replacement, especially not for Gram-negative bacteria.

Regarding phages, where most of the work has been performed in Eastern Europe and the states of the former Soviet Union (25), they too have their advantages as well as significant drawbacks. Phage therapy can be useful in e.g. deep skin wounds, where it is possible to spray the phage solution directly onto the infected area. However, caveats that would hinder the use of phages systemically is their considerable antigenicity, localization issues with getting the phage to the infected site, achieving the appropriate concentration, as well as a need constantly to update phage libraries to get a useful coverage of strains. In addition to this, bacteria can quite easily develop resistance also to phages (26).

In summary, alternative approaches (and there are many more beside AMPs and phages) to conventional antibiotics should certainly be explored, but it is important clearly to understand their limitations in terms of which types of infections and bacteria they could be effective against, and how close they are to clinical application.

Box 1. Case study of Ramoplanin™ with relevant dates tracking the discovery, patent, out-licencing, and changing ownership of the molecule.

1980	Patent, Gruppo Lepetit SpA/Aventis
1984	First published paper
1996	Buyout of Lepetit from Aventis by Biosearch Italia
1997–2001	More patents taken by Biosearch
1999	Intrabiotics starts phase II studies
2001	Presentation of *in vitro* data (*C. difficile*, VRE) at ICAAC
2001	Ramoplanin gets EMA Orphan drug designation
2001	Biosearch reacquires rights to oral Ramoplanin from Intrabiotics. Intrabiotics had completed phase II trials but failed to recruit patients for phase III (104/950 needed)
2001	Genome Therapeutics acquires licence for oral (not topical) Ramoplanin and planned to complete phase III trial
2002	Biosearch reacquires rights to topical Ramoplanin from Intrabiotics
2002	Versicor buys Biosearch
2003	Versicor changes name to Vicuron
2004	Genome Therapeutics gets FDA fast-track status for Ramoplanin
2004	Genome Therapeutics changes name to Oscient
2005	Pfizer buys Vicuron
2005	Oscient gets Special Protocol Assessment from the FDA, needs to complete 2 phase III trials for VRE
2006	Oscient acquires world-wide rights for Ramoplanin from Pfizer/Vicuron
2007	Oscient receives Notice of allowance from the FDA for methods of use for *C. difficile*
2009	Oscient declares bankruptcy
Dec 2009	Nanotherapeutics acquires rights to Ramoplanin

Conclusions and proposals

To accomplish any of the suggestions in this paper, new funding is essential. The Wellcome Trust funds both academia and companies for early drug discovery/screening of compounds to try and close the gap between academia and commercialization in their Seeding Drug Discovery programme (£110 million), but funding is mainly available for partners in the UK. In a 255-page report for the Swedish Presidency of the EU in 2009 intended to investigate how to make possible the continued development of innovations in the antibacterial field, some suggestions are made: To create a collaborative programme, in order to collect the expertise that is required to develop innovations in the antibacterial field, and to provide public risk capital for early, high-risk antibiotic development (27).

There is an increasing interest and investment in translational medicine, where lessons could be learned in order to cross the 'valley of death' in antibacterial research. Examples include National Center for Advancing Translational Sciences (NCATS) at NIH[7] and other initiatives in the UK,[8] India,[9] and many other places. In order to meet the numerous challenges listed in this paper—

[7]http://www.ncats.nih.gov/

[8]http://www.mrc.ac.uk/Ourresearch/ResearchInitiatives/Translationalresearch/index.htm

[9]http://thsti.res.in/

Box 2. Proposed research questions that could be explored to aid easier antibiotic discovery.

The entry/exit problems

- Methods to measure entry of molecules into (Gram-negative) bacteria are needed—there are some tentative thoughts that mass spectrometry may be a way forward, but the methods are a long way from being truly useful (28)
- Develop rules, like an antibacterial version of 'Lipinsky's rule of 5′ for cytoplasmic entry into Gram-negative bacteria
- Studies of self-promoted uptake, including investigating more closely the surface structure and charge of the outer membrane in Gram-negatives
- Investigate more closely which properties that make molecules substrate for efflux pumps

Chemical novelty

- Better screening libraries are needed, keeping in mind that antibacterials in general have been found to have differing chemical properties than other pharmaceuticals (29). Maybe virtual screens of compounds, e.g. in PubChem, could be useful as a first step, much like that performed at the University of Dundee for neglected diseases (30)
- Create new, more varied libraries—however, diversity can mean many different things, and it needs to be defined how best to achieve diversification
- New libraries could also be created by going back to natural products and finding new sources of compounds such as slow-growing micro-organisms, marine bacteria, or products of cryptic pathways. The molecules need to be coupled with whole-cell screens and assays that allow for effective de-replication of already discovered natural products (31)

Avoiding resistance

- Increase fundamental knowledge of under what circumstances (clinically relevant) resistance mutations arise
- Finding ways of 'reversing' resistance other than beta-lactamase and efflux pump inhibitors, such as perhaps inactivating the Cfr-methylase that gives resistance to a variety of ribosome-directed drugs
- Better animal models are needed, to understand better the correlation between *in vitro* and *in vivo* resistance data. Tests for efficacy usually involve a loading dose of ~10^3–10^5 bacteria, so resistance events, occurring a frequency of 1 in 10^7 or less, may rarely be picked up

Other

- Methods to test the usefulness of anti-virulence approaches *in vivo*, perhaps by using engineered strains that can turn off virulence factors during an infection
- Investigate which panel(s) of strains that are the most relevant to test new antibiotics against
- Address the lack of structural data for many bacterial proteins

scientific bottlenecks, mechanisms for prioritization, lack of innovative capacity, misaligned incentives, and lack of antibiotic discovery experience and expertise—we need a strong focal point with multidisciplinary skills, and both academic and industry competences. I propose the establishment of a 'Centre for Antibiotic Research', much as we have institutes and centres for cancer research, to help address these challenges and also help create clear career paths for scientists in antibacterial discovery. A model could be Canada's Center for Drug Research and Development[10] that provides expertise and infrastructure for researchers, to enable them to advance promising drug candidates.

A possibility for Europe could be to create such a centre within the framework of the JPIAMR, to collect Europe's (and Canada's) expertise in antibiotic discovery. The centre could have outreach programmes to collect interesting molecules and offer expertise in drug development, to enable evaluation of potential antibiotic compounds originating in academia or smaller biotech companies. However, it is high time that we stop just discussing potential solutions, find the necessary funding, and start implementing.

[10]http://www.cdrd.ca/

Acknowledgements

The author would like to extend her gratitude to all the researchers who have willingly answered my questions, with a special thanks to Lynn Silver, for her time and willingness to share her expertise, and the JPIAMR for introducing me to researchers for interviews. The author has been supported by a grant from the Kjell and Märta Beijer Foundation.

Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

References

1. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. Lancet Infect Dis. 2013;13:1057–98.
2. Theuretzbacher U. Accelerating resistance, inadequate antibacterial drug pipelines and international responses. Int J Antimicrob Agents. 2012;39:295–9.
3. Freire-Moran L, Aronsson B, Manz C, Gyssens IC, So AD, Monnet DL, et al. Critical shortage of new antibiotics in development against multidrug-resistant bacteria-Time to react is now. Drug Resist Updat. 2011;14:118–24.
4. Silver LL. Challenges of antibacterial discovery. Clin Microbiol Rev. 2011;24:71–109.

5. Rex JH, Eisenstein BI, Alder J, Goldberger M, Meyer R, Dane A, et al. A comprehensive regulatory framework to address the unmet need for new antibacterial treatments. Lancet Infect Dis. 2013;13:269–75.
6. Mossialos E, Morel CM, Edwards S, Berenson J, Gemmill-Toyama M, Brogan D. Policies and incentives for promoting innovation in antibiotic research. 2010. Available at http://www.euro.who.int/en/about-us/partners/observatory/studies/policies-and-incentives-for-promoting-innovation-in-antibiotic-research. Accessed 17th december 2013.
7. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov. 2007;6:29–40.
8. So AD, Gupta N, Brahmachari SK, Chopra I, Munos B, Nathan C, et al. Towards new business models for R&D for novel antibiotics. Drug Resist Updat. 2011;14:88–94.
9. Kesselheim AS, Outterson K. Fighting antibiotic resistance: marrying new financial incentives to meeting public health goals. Health Aff (Millwood). 2010;29:1689–96.
10. Nathan C. Aligning pharmaceutical innovation with medical need. Nat Med. 2007;13:304–8.
11. ReAct. Collaboration for innovation. 2011. Available at http://www.reactgroup.org/uploads/resources/Collaboration-for-Innovation-for-webb.pdf. Accessed 17th december 2013.
12. So AD, Ruiz-Esparza Q, Gupta N, Cars O. 3Rs for innovating novel antibiotics: sharing resources, risks, and rewards. BMJ. 2012;344:e1782.
13. WHO. Global incidence and prevalence of selected curable sexually transmitted infections. 2008. Available at www.who.int/reproductivehealth/publications/rtis/2008_STI_estimates.pdf. Accessed 17th december 2013.
14. Swedish Government. Innovative incentives for effective antibacterials. 2009. Available at http://www.reactgroup.org/uploads/what-we-do/innovative-incentives-for-effective-antibacterials.pdf. Accessed 17th december 2013.
15. Council of the European Union. Council Conclusions on innovative incentives for effective antibiotics. 2009. Available at http://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/lsa/111608.pdf. Accessed 17th december 2013.
16. European Commission. Communication from the Commission to the European Parliament and the Council Action plan against the rising threats from antimicrobial resistance. 2011. Available at http://ec.europa.eu/dgs/health_consumer/docs/communication_amr_2011_748_en.pdf. Accessed 17th december 2013.
17. Bergstrom R. The role of the pharmaceutical industry in meeting the public health threat of antibacterial resistance. Drug Resist Updat. 2011;14:77–8.
18. Sams-Dodd F. Is poor research the cause of the declining productivity of the pharmaceutical industry? An industry in need of a paradigm shift. Drug Discov Today. 2013;18:211–17.
19. White RJ. The early history of antibiotic discovery: empiricism ruled. In Dougherty TJ, Pucci MJ, editors. Antibiotic discovery and development. Springer; 2012.
20. Innovative Medicines Initiative. 2012. Available at http://www.imi.europa.eu/sites/default/files/uploads/documents/6th_Call/1_Annex%201%20%206th%20Call%20for%20Proposals_2012%2005%2024.pdf. Accessed 17th december 2013.
21. Roy A, McDonald PR, Sittampalam S, Chaguturu R. Open access high throughput drug discovery in the public domain: a Mount Everest in the making. Curr Pharm Biotechnol. 2010;11:764–78.
22. Manchester JI, Buurman ET, Bisacchi GS, McLaughlin RE. Molecular determinants of AcrB-mediated bacterial efflux implications for drug discovery. J Med Chem. 2012;55:2532–7.
23. Silver LL. Antibacterial discovery: problems and possibilities. In Gualerzi C, Brandi L, Fabbretti A, Pon CL, editors. Antibiotics: targets, mechanisms and resistance. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2013.
24. Hancock RE, Nijnik A, Philpott DJ. Modulating immunity as a therapy for bacterial infections. Nat Rev Microbiol. 2012;10:243–54.
25. Kutateladze M, Adamia R. Phage therapy experience at the Eliava Institute. Med Mal Infect. 2008;38:426–30.
26. Fernebro J. Fighting bacterial infections—future treatment options. Drug Resist Updat. 2011;14:125–39.
27. VINNOVA. Bad Bugs—Future Drugs? 2009. Available at http://www.vinnova.se/upload/Bad%20Bugs%20Future%20drugs.pdf. Accessed 17th december 2013.
28. Hrabak J, Chudackova E, Walkova R. Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. Clin Microbiol Rev. 2013;26:103–14.
29. O'Shea R, Moser HE. Physicochemical properties of antibacterial compounds: implications for drug discovery. J Med Chem. 2008;51:2871–8.
30. Brenk R, Schipani A, James D, Krasowski A, Gilbert IH, Frearson J, et al. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. ChemMedChem. 2008;3:435–44.
31. Singh SB, Young K, Miesel L. Screening strategies for discovery of antibacterial natural products. Expert Rev Anti Infect Ther. 2011;9:589–613.