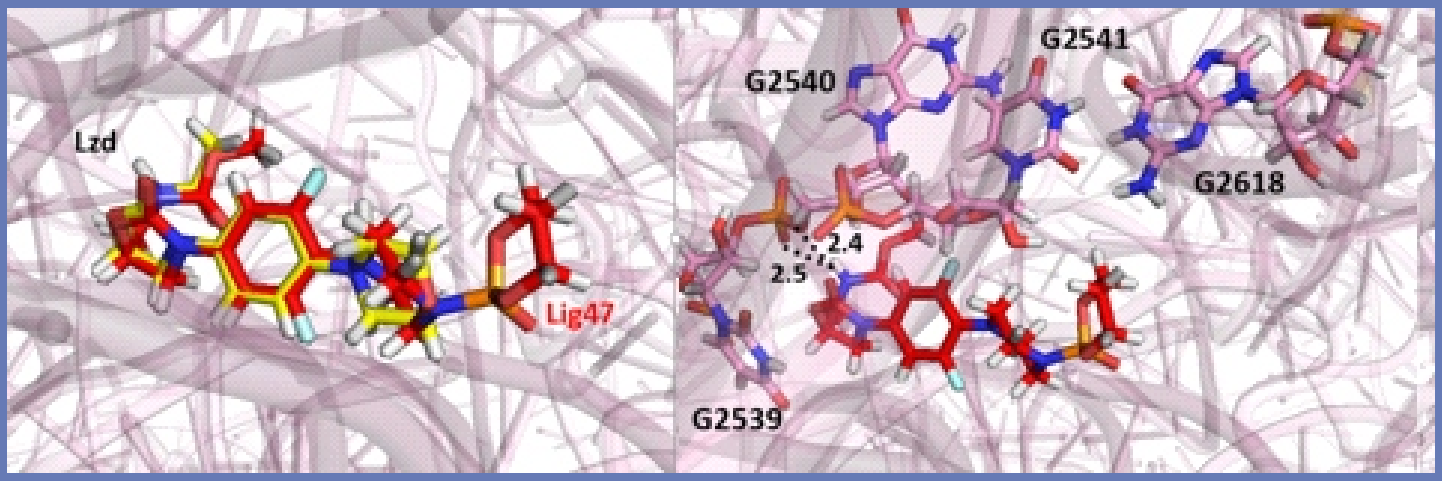
ISSN 0972-589X

CRIPS

Vol. 17, No. 1, January-February 2023

Current Research & Information on Pharmaceutical Sciences



Novel Phosphorous Containing Oxazolidinones

Use of persulfate beyond organic chemistry

Drug Metabolism: Pharmacoinformatics efforts

CRIPS Digest

National Institute of Pharmaceutical Education and Research (NIPER)
S.A.S. Nagar

S.A.S. Nagar

Guidelines for the preparation of manuscripts

Original work as a **Regular Article** or **Rapid Communication** must be presented by the author. For publication, topics related to **Reviews** of important fields, **Perspective Articles** in emerging areas and comment articles on recently published articles (a part of CRIPS digest) will also be considered for submission. **Expert opinion articles** will include only the invited articles. The novelty of the work and names of three potential referees must be included by the author in the cover letter. All articles will be subjected to peer review. The manuscript submitted by the author will only be considered in case if it has not been published in the past and has not been put forward for publication elsewhere and moreover, if the manuscript is accepted then it will not be published elsewhere.

Typescript

Manuscript submitted by the author should be prepared in grammatically correct English and free from plagiarism issues. Preferred file for the preparation is Microsoft Word (.doc or .docx) or LATEX with 2.5 cm margin on all sides and a line spacing of 1.5 cm. Figures, schemes and tables must contain captions and should be incorporated at appropriate places in the text having distinguishable separation from the main text.

(1) Short and self-explanatory title of the paper is preferred.

(2) Manuscript must contain the name of all author(s) with initial(s) and the name and address of the institution where the work was carried out. Author may provide present address in case if different from the above along with the email-id and phone number of the corresponding author.

Abstract and Keywords

Abstract of all papers should not exceed the word limit of 200. Numerical results should not be presented and avoided as far as possible. Graphical abstract consisting of synopsis (word limit of 50) along side a suitable figure or equation will be required on the acceptance of the manuscript. 4-6 keywords must be provided.

Graphical abstract must be provided by the author for the 'Table of Contents' page.

Graphical abstract page should incorporate, (a) a synopsis of the paper (not more than 50 words) and (b) PICTOGRAM: one (only one) important small figure/photograph (above mentioned abstract should be prepared carefully to avoid the loss of clarity and legibleness of the text when reduced to a size of 7 cm x 5 cm).

Text

The main five sections of the articles must be divided as: (i) Introduction, (ii) Experimental or Theoretical/Computational, (iii) Results and Discussion,(iv) Conclusions and (v) References. Numbering of main sections should be done as 1,2 *etc.,* sub sections as 1.1, 1.2 *etc*., and further as 1.1.1, 1.1.2 *etc*. SI system of units and symbols is recommended for use. The division of article into four sections is not applicable to Reviews, Perspectives and Rapid Communications.

Tables

Consecutive numbering of all the tables in Arabic numerals in the order of their appearance in the text is mandatory. Tables should be self-contained and have a descriptive title.

Figures

Photographic figures should be numbered consecutively in Arabic numerals in the order of their appearance in the text. Each figure must contain captions separately. Software like ChemDraw, IsisDraw *etc*. should be used for line drawings and structures. Lines should be made sufficiently thick in a way for reduction to a half or third of the original size. All figures must be original. If the reproduced from other sources, appropriate permission for reproduction should be obtained, the same should be mentioned in the figure caption.

Symbols and Mathematical material

Before submitting the manuscript as well as the pdf file prepared by the Editorial Manager, author should carefully check all the symbols and equations for correction. All equations must be numbered consecutively in Arabic numerals. Equations should be clearly written with each one a separate line and well separated from the text. Special characters (Greek letters, vector, tensor, matrix, symbols, etc.) should be in bold and carefully checked by the author before submission. In case of extensive use of symbols, author should provide an explanatory table of the symbols on a separate page.

References

References should be listed at the end of the manuscript in serial order and in a text as a superscript number in order of appearance. The format for references is as follows:

Journals (Note: Cite either page number or Article ID number, as appropriate): Desiraju G R 1991 The C-H... O Hydrogen Bond in Crystals: Whatis it? Acc. Chem. Res. 24 290

Wagh R B, Gund S H and Nagarkar J M 2016 An eco-friendly oxidation of sulfide compounds. J. Chem. Sci. 128, 1321

Books: Tauber M J, Tauber C A and Masaki S (1986) Seasonal Adaptations of Insects, Oxford University Press, NY, US

Supplementary Information (SI)

In supplementary information, extensive Data/ Figures/Tables and scanned copies of recorded spectra can be submitted by the author. It is mandatory to submit scanned copies of one or more of the following spectra/reports, as applicable: NMR (^{1}H, ^{13}C) spectra; ESR spectra; cif (converted to Microsoft Word file), Check CIF files and CCDC deposition numbers, but NOT Fo/Fc Tables for crystal structure analysis; mass spectra; elemental analysis reports; etc. For newly synthesized molecules. Labeling of figures and tables in Supplementary Information should be done as S1, S2 etc.

All the content of Supplementary Information is to be stated briefly in a paragraph in the manuscript after 'Conclusions'.

Acknowledgments are optional.

Submission

By e-mail to: crips@niper.ac.in in both MS word (.doc/.docx) and .pdf formats. Please note the editorial changes may be made to fit to chosen pages. The same needs to be confirmed by the authors during proof reading.

Payment Modes for CRIPS Subscription & Advertisement

Option 1 **Make a draft in the Name of:**
National Institute of Pharmaceutical Education and Research (NIPER),
Sector 67, S.A.S. Nagar (Mohali), PIN - 160062, Punjab (India)

Send the Draft to:
Dr. Vishnu Kumar Sharma, CRIPS, Room Number 210, Block-A, NIPER,
Sector 67, S.A.S. Nagar (Mohali), PIN - 160062, Punjab (India)
*Send a copy of the draft to crips@niper.ac.in along with postal details.

Option 2 **Via Net Banking - (NEFT/RTGS)**

Account Number	55034549623
IFSC Code	SBIN0004421
Bank Name	State Bank of India
Branch Name	SPL Housing Finance
Complete Branch Address	SPL Housing Branch, SCF-32, Phase - 10, S.A.S. Nagar, Punjab, India
MICR Number	160002034
Account Type	Saving Bank Account

Option 3 **UPI Payment (Scan and Pay)**

Scan and Pay/UPI Payment

As soon as the electronic transfer is done, please send an email (crips@niper.ac.in) giving the following details: (a) Date of transfer (b) Amount(c) Name of your Bank/Branch (d) Transaction Reference No. given by your bank and (e) Postal details for further communication. Similarly, send a copy of the draft to crips@niper.ac.in

For any further query related to Subscriptions & Advertisements, you may also mail us at crips@niper.ac.in, cripsbuiness@niper.ac.in

Subscription & Advertisement Charges

Charges for advertisement

Rs. 15,000/- for backside cover full size (in multicolor)
Rs. 10,000/- for inside cover full size (in multicolor)
Rs. 7,500/- for inside cover half size (in multicolor)
Rs. 5,000/- for inside CRIPS full page (in two colors)
Rs. 3,000/- for inside CRIPS half page (in two colors)

Charges for subscription

Rs. 1,200/- for individuals and colleges/institutions
Rs. 2,500/- for industrial subscribers

13th Convocation of NIPER, S.A.S. Nagar

NIPER Foundation Day 2023

CRIPS Current Research and Information on Pharmaceutical Sciences

Vol. 17, No. 1, January-February 2023

BUSINESS CORRESPONDENCE

Enquiries concerning advertisements should be addressed to the Editorial Office CRIPS.

Published by National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar (Punjab)

EDITORIAL OFFICE

National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar - 160062 (Punjab), INDIA

Tel. : 0172-2292000,2214682
E-mail : crips@niper.ac.in
web : www.niper.gov.in

The cover page contains a figure from the article of Dr. Jagattaran Das

EDITORIAL

Antimicrobial resistance (AMR) has been declared as a global health threat by world health organization (WHO). The misuse, overuse of antimicrobials are the major causes for the development of resistance. There is an urgent need for development of new antimicrobials against resistant microbes. The oxazolidinones are potent antibacterial agents effective against multidrug-resistant Gram-positive bacteria. Linezolid is the first generation oxazolidinone antibacterial agent. However, microbes developed resistance to Linezolid after few years and it led to development of next generation oxazolidinones. Tedizolid is effective against skin infection, and Radezolid is in clinical trial for treatment of infection caused by Linezolid resistant microbes. The phosphorous-phenyloxazolidinones have been found to inhibit the linezolid resistant microbes. The compounds were designed by molecular docking studies, synthesized and found active against linezolid resistant microbes in in-vitro studies.

The persulfates such as sodium persulfate and potassium persulfate are used as oxidants for C-C C-X bond formation reactions, and an initiator for polymerization reactions for manufacturing of polymers. Persulfates have significant applications in biological and drug degradation studies. Potassium persulfate has been employed in the preparation of an artificial red blood substitute (grafted starch-encapsulated haemoglobin). Potassium persulfate is utilized as a disinfectant in livestock, aquaculture and poultry products. Potassium persulfate acts as an electron acceptor in for photo catalytic degradation of metformin, and other organic pollutants. Persulfates are safe organic reagents, which have broad applications.

Drug metabolism is a pharmacokinetic process which facilitates the drug's effectiveness and safe elimination from the body. The drugs are metabolized by enzymes or xenobiotics, such as by the CYP450 family of enzymes. The Phase I metabolism process involves oxidation, reduction, and hydrolysis reactions. The drugs may require further modifications (phase II metabolism) which involves conjugation reactions. In a few cases, the metabolism process can generate reactive metabolites and cause toxicity. The in silico tools are utilized to predict, investigate the biotransformation in human body. Artificial intelligence (AI) methods are being developed to study drug metabolism and associated toxicities. Quantum chemistry has been applied to investigate drug metabolism and toxicity. Quantum chemical analysis predicted the atomic-level information about the metabolism of familiar antiviral drug remdesivir. Chemoinformatics studies, Quantum chemical studies and AI are considered as potential tools for prediction of drug metabolism and toxicities.

Dr. Sharada Prasanna Swain

Research Article

Synthesis, SAR and Biological Evaluation of Novel Phosphorous Containing Oxazolidinone Derivatives as Antibacterial Agents

Jagattaran Das[a*], Sandeep Kanwar[a], Mini Bhutani[a], Rishi Sharma[a], Gurpreet Singh[a], Anoop Tanwar[a], Vishal Sudan[a], Shweta Kapoor[a], Shailender Chauhan[a], Tamanna Toor[a], Sandeep Kaur[a], Baldev Gautam[a], Aabid A. Wani[b], Sudhir K. Sharma[a], M. Sitaram Kumar[a], Sanjay Trehan[a] and Prasad V. Bharatam[b*]

[a] Drug Discovery & Research, Panacea Biotec Ltd., Ambala-Chandigarh Highway, Lalru-140501, Punjab, India, *E-mail: jagat30@hotmail.com,
[b] Dept. of Medicinal Chemistry, National Institute of Pharmaceutical Education & Research (NIPER), SAS Nagar (Mohali), Punjab - 160062, India, *E-mail: pvbharatam@niper.ac.in

There is an unmet medical need to discover and develop innovative antibacterial compounds active against resistant Gram-positive bacteria. A series of novel oxazolidinone analogues having phosphorous substitution were synthesized and their antibacterial activities evaluated against a panel of resistant and susceptible Gram-positive bacteria. Potent *in vitro* antibacterial activities were exhibited by several compounds against all the organisms tested including linezolid resistant strains. To the best of our knowledge, this is the first report of phosphorous substituted phenyloxazolidinones. One of the synthesized compounds, compound **47**, has been shortlisted for further evaluation. Additionally, molecular docking studies suggest that compound **47** has additional interactions with ribosome than that of linezolid in 50S RNA.

INTRODUCTION

The emergence of bacterial resistance to existing antibiotics has become a problem worldwide.[1] Of particular concern are infections caused by multidrug-resistant gram-positive pathogens primarily methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis (MRSE), Vancomycin-resistant Enterococcus faecium (VREF) and Glycopeptide Intermediate Resistant Staphylococcus aureus(GISA).[2-5] The alarming rate of resistance development has prompted scientists to explore novel antibacterials active against multi-drug resistant Gram-positive bacterial pathogens. The oxazolidinones, a class of synthetic antibacterial agents, are found to be active against multidrug-resistant Gram-positive bacteria that are resistant to other clinically useful antibiotics.[6-12] Linezolid **1** (Figure 1), launched by Pharmacia having the trade name Zyvox®, represents the first member of this class to receive regulatory approval. Unfortunately, within a few years, resistance against linezolid has surfaced particularly in Enterococcus faecium and in *S. aureus* strains.[13]

The discovery of linezolid and limitations associated with it has spurred intense research efforts directed towards the development of novel oxazolidinone antibacterials.[14-18] Linezolid was launched in 2000.

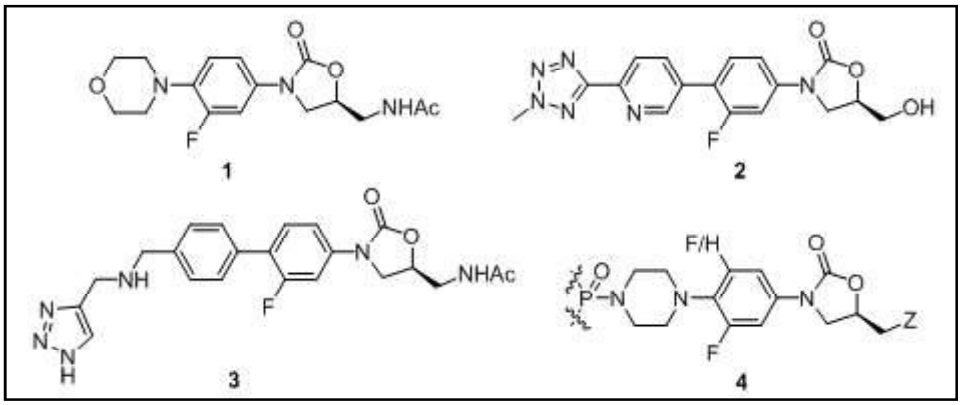
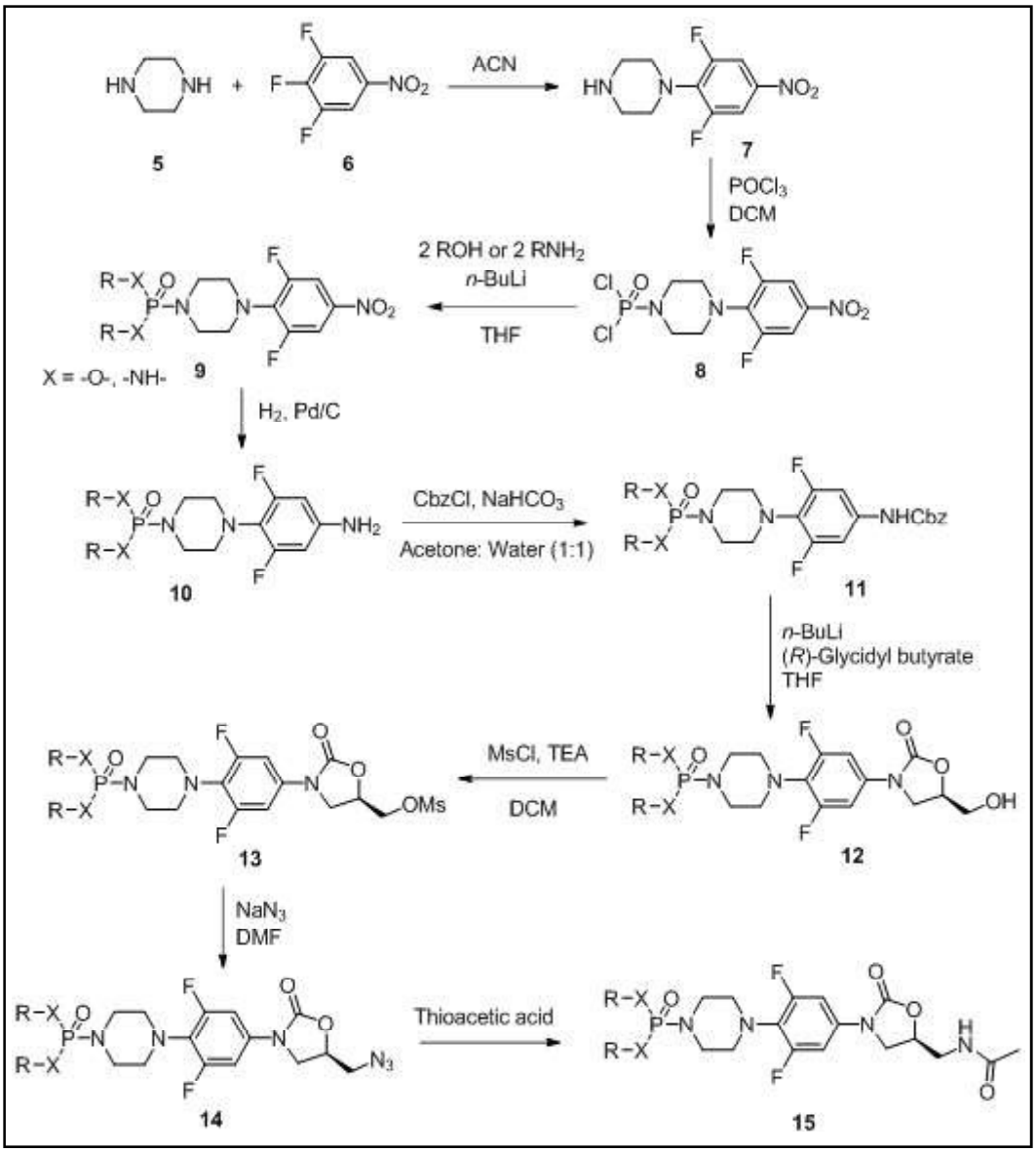


Figure 1: Oxazolidinone based antibacterials.

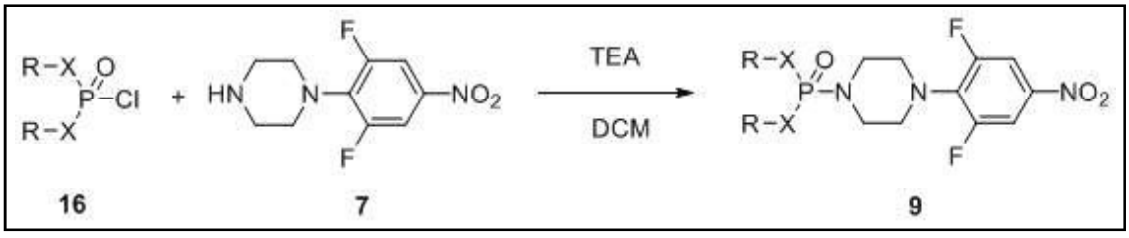


Scheme 1: Synthesis of acetamide derivatives of phosphorous substituted oxazolidinones.

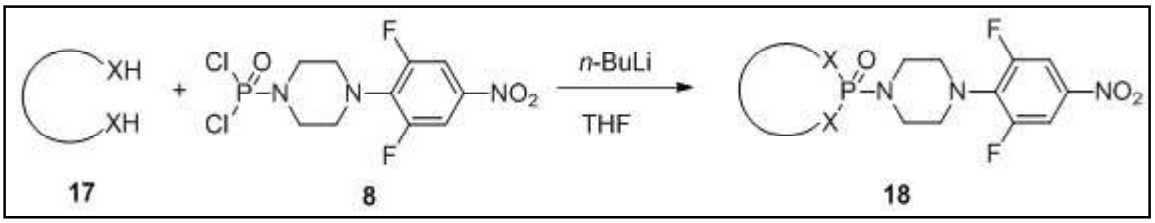
However, it took 14 years for the second molecule from oxazolidinone series, Tedizolid **2** (effective against skin infection), to come to the market and Radezolid **3** (active against LNZ resistant strains), the third molecule, is in late stage of clinical trial. Linezolid is widely employed for treating infections caused by Gram-positive bacteria. It is also effective in the treatment of drug-resistant pulmonary infections and multidrug resistant TB (MDR-TB). Many other oxazolidinones are at different stages of development (sutezolid, eperezolid, delpazolid, TBI-223) and some of them are being developed against MDR-TB. It may be mentioned here that, according to a 2014 data, newly diagnosed TB patients (about 3.3%) and previously treated TB patients (about 20%) are reported to have MDR-TB.[19]

Making structural modifications in the existing antibacterial drugs seems to be the most common approach to develop new antibacterial agents to provide novel analogues with improved biological profile.[17] We found that oxazolidinone compounds **4** (Figure 1) with the special phosphorous substitution have not been explored till now for their antibacterial acitivity.[20] We envisaged, these phosphorous containing substituents might improve activity compared to linezolid and in turn might overcome resistance associated with it. To check this, molecular docking studies were carried out using the crystal structure of 50S ribosome unit of E. coli (PDB ID: 3CPW).[21] It was observed that both linezolid and novel compounds **4** bind in the same pocket of ribosome. Interestingly, phosphorous substitution played an important role in addition to the oxazolidinone ring in binding to 50S RNA. Keeping this in mind, various oxazolidinone derivatives with phosphorous substitutions were synthesized following Scheme 1.

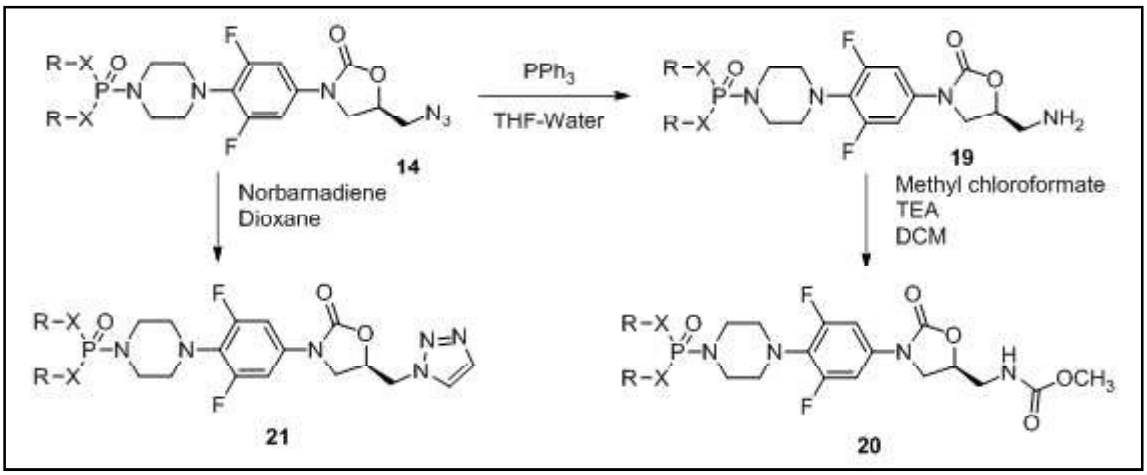
Reaction of piperazine **5** with 3,4,5-trifluoronitrobenzene **6** in acetonitrile provided nitro compound **7** as orange solid in 92% yield. Nitro compound **7** was reacted with phosphoryl chloride



Scheme 2: Alternate synthetic route for the generation of the **9**.



Scheme 3: Synthesis of cyclic phosphorous derivatives.



Scheme 4: Synthesis of carbamate and triazole derivatives.

under nitrogen atmosphere in DCM to provide phosphonic dichloride 8 as yellow solid in very good yield (70%). This novel dichloro compound is stable and can be stored under nitrogen atmosphere. Phosphonic dichloride **8** was then treated with **2** equiv. of appropriate alcohol or amine to obtain compounds **9** as yellow solids (70-80%). Nitro derivatives **9** were then reduced by hydrogenation over palladium catalyst on activated charcoal to give compounds **10** as white solid in 90-95% yield. Amines **10** upon subsequent reaction with CbzCl, in the presence of sodium bicarbonate afforded compounds **11** as white solids (90-95% yield). Oxazolidinone ring was then constructed by deprotonating **11** using n-butyllithium in THF followed by the addition of (R)-glycidyl butyrate to produce the oxazolidinones **12** (off-white solid) in 60-70% yield. Compounds **12** were reacted with methanesulfonyl chloride in DCM to afford mesylates **13** as off-white solid (90-95%). Reaction of mesylates **13** with sodium azide in DMF gave azides **14** as off-white solid in 80-85% yield. The desired acetamides **15** were prepared by reaction of azides **14** with thioacetic acid. The final compounds were mostly white solids and were obtained in 75-85% yield.

Some of the nitro derivatives **9** were prepared following Scheme 2. Commercially available compounds **16** were reacted with compound **7** using triethylamine as base in DCM to give nitro derivatives **9** as yellow solid in 85-90% yield. These nitro derivatives **9** were then converted to desired oxazolidinones **15** following procedure outlined in Scheme 1.

The cyclic phosphorous derivatives were prepared following Scheme 3. Diols or diamines **17** were reacted with dichloro derivative **8** to obtain nitro derivatives **18** as yellow solid in 70-80% yield. These nitro derivatives **18** were then converted to desired acetamide oxazolidinone antibacterial compounds following procedure outlined in Scheme 1.

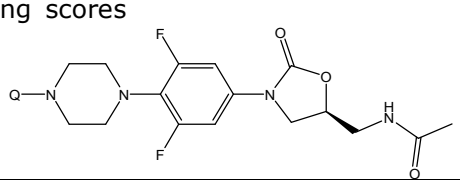
Carbamate derivatives **20** and triazole derivatives **21** were prepared following Scheme 4. Azides **14** were reduced to amines 19 using triphenyl phosphine in THF-water in 75-80% yield. Carbamate derivatives **20** were prepared by reacting amine **19** with methyl chloroformate in the presence of triethylamine as base in DCM at 0 °C. Carbamates were obtained as white solid in 90-95% yield. Triazole derivatives 21 were prepared by reacting azide derivatives **14** with norbornadiene in dioxane at 80°C as white solid (70-75%).

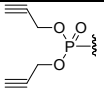
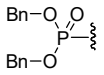
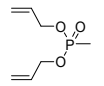
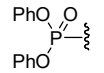
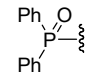
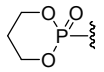
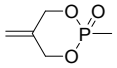
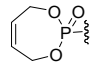
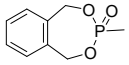
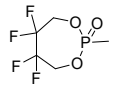
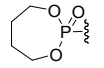
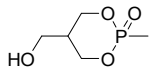
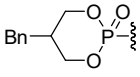
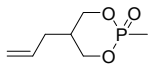
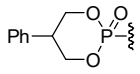
Triazole derivatives **21** were alternatively prepared by reacting mesylate **13** with potassium salt of 1,2,3-triazole in 40-45% yield.

Thus phosphorous containing oxazolidinones with various substituents either attached directly or via oxygen or nitrogen, were synthesized and screened for their in vitro antibacterial activities against a panel of resistant and susceptible Gram-positive bacteria. The results of the acetamide series are summarized in Table 1.

Most of the targeted compounds displayed very good in vitro antibacterial activities. As shown in Table 1, only a few open chain compounds **22-26** showed activities comparable to linezolid whereas almost all cyclic compounds **27-43** showed activities either comparable to or better than linezolid. All the compounds linked either through oxygen or directly through carbon showed potency equal to or better than linezolid, whereas nitrogen linked compounds

Table 1: In vitro (MIC, μg/ml) activity of novel acetamide oxazolidinones and their docking scores



Compound No.	Q	*S. aureus* ATCC 29213	*S. aureus* ATCC 33591	*E. faecalis* ATCC 29212	*E. faecium* ATTC 700221	*Docking score*
22		2	1	2	1	-7.47
23		4	2	2	1	-7.67
24		4	2	2	2	-6.85
25		2	2	2	2	-6.84
26		2	2	2	2	-5.99
27		2	2	2	2	-7.83
28		2	1	2	2	-7.61
29		2	1	1	2	-7.74
30		4	2	2	4	-7.64
31		2	1	1	2	-7.82
32		2	2	2	4	-7.34
33		4	4	2	4	-7.31
34		4	2	2	2	-6.73
35		4	1	2	2	-7.90
36		4	4	2	2	-6.92

44-46 showed weak/no antibacterial activities. Based on the activities of Table 1, a few compounds were chosen for further modification (replacement of acetamide group with methyl carbamate or 1,2,3-triazole) and their results are summarized in Table 2.

From Table 2, we can see that carbamate and triazole derivatives were either similar or more potent than their corresponding acetamides. Almost all the synthesized compounds showed antibacterial activities either comparable to linezolid or 2-4 times more potent than linezolid.

In the present series under investigation, difluoro derivatives were more effective than their corresponding monofluoro derivatives. This is evident from Table 3 (compare compounds **41** with **69, 51** and **70, 60** and **71**)

As discussed earlier, LRSA strains have started to develop resistance in clinical conditions and the next generation oxazolidinones are expected to possess activity against LRSA strains. A few oxazolidinone compounds from the present series were screened for in vitro activity against in house developed LRSA strain of ATCC 25923 and results are summarized in Table 4.

As evident from Table 4 most of these oxazolidinones showed improved activity against LRSA strain of ATCC 25923. Moreover, compounds **40, 41, 47, 58, 60, 66** were found to be 8 fold more potent than linezolid against LRSA strain of ATCC 25923.

Our next aim was to assess pharmacokinetic (pk) performances of these novel compounds. A Few compounds were selected for this purpose whose PK efficacies were checked in Swiss Albino mice by single dose (30 mg/kg) using oral route of administration and results are summarized in Table 5. From Table 5, it is evident that almost all selected compounds showed excellent

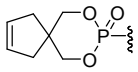
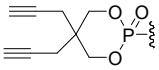
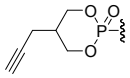
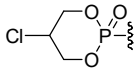
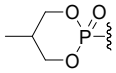
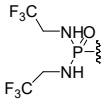
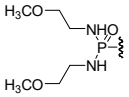
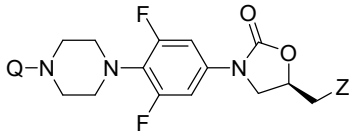
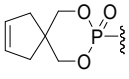
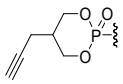
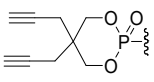
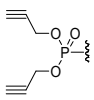
39		4	2	2	2	-7.61
40		2	1	1	2	-6.98
41		2	2	2	2	-7.58
42		4	2	2	2	7.59
43		4	2	2	2	-7.77
44		64	64	16	16	-7.91
45		64	128	32	64	-6.92
46		18	8	8	16	-7.58
1	Linezolid	2	1	2	2	**-8.32**

Table 2: In vitro (MIC, µg/ml) activity of novel oxazolidinones and their docking scores



Compound No.	Q	Z	*S. aureus* ATCC 29213	*S. aureus* ATCC 33591	*E. faecalis* ATCC 29212	*E. faecium* ATTC 700221	*Docking Score*
47		-NHCOOCH$_3$	4	2	2	2	**-8.18**
48		-NHCOOCH$_3$	2	1	2	1	-7.93
49		-NHCOOCH$_3$	2	1	1	1	-7.45
50		-NHCOOCH$_3$	4	4	4	4	-7.14
51		-NHCOOCH$_3$	1	1	1	1	-6.59
52		-NHCOOCH$_3$	1	1	1	1	-7.70
53		-NHCOOCH$_3$	2	4	4	4	-7.46

PK profile, better than linezolid in most cases. The carbamate series possessed best PK profile followed by acetamide series. Triazole compounds had PK similar to linezolid or bit inferior (Compare **27, 47** and **58**). Based on the in vitro activities and PK data, compounds having activity similar or superior to linezolid were subjected to in vivo studies in systemic infection model induced by S. aureus ATCC 29213. ED50 values were calculated after oral administration of compounds in Swiss Albino mice. Most of the compounds showed ED_{50} of ~10, the most active compound **47** showed ED50 value of 9.

To understand the observed activity of compound **47** (especially 8-fold better activity in LRSA strain compared to linezolid) and its important interactions in the active site of ribosome, molecular docking studies of compound **47** was performed in relation to linezolid using Glide software.[22] Figure 2a shows the superimposition of linezolid and compound **47** in the corresponding docking pose. Figure 2b shows the interaction between compound **47** and the ribosome.

The most important interactions of compound **47** with 50S ribosome unit are- i) Hydrogen bonding interactions of NH group (carbamate) of compound **47** with G2540 (estimated bond length 2.4 Å); ii) Hydrophobic interactions between difluoro phenyl group of compound **47** with U2541; iii) the oxazolidinone ring of compound **47** is stabilized through hydrophobic interaction with U2539 and iv) The cyclic phosphoric ester of compound **47** is stabilized through hydrophobic

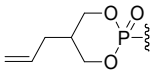
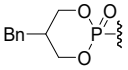
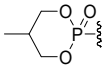
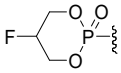
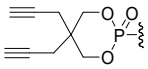
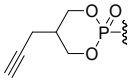
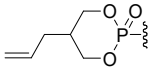
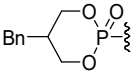
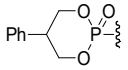
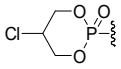
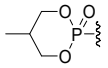
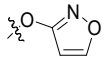
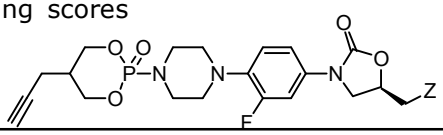
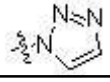
54		-NHCOOCH$_3$	4	2	2	2	-7.61
55		-NHCOOCH$_3$	4	2	2	2	-7.58
56		-NHCOOCH$_3$	4	2	2	1	7.70
57		-NHCOOCH$_3$	2	2	2	1	-7.75
58			2	1	1	1	-7.38
59			2	2	2	2	-7.80
60			2	1	1	2	-6.53
61			2	1	2	1	-7.57
62			4	2	4	2	-5.14
63			2	1	2	0.5	-7.55
64			2	1	2	1	-7.50
65			2	0.5	2	1	-4.66
66			1	1	1	1	-7.52
67			4	2	4	2	-7.22
68			2	2	2	1	-6.26
1	Linezolid		2	1	2	2	-8.32

Table 3: In vitro (MIC, µg/ml) activity of novel monofluoro oxazolidinone and their docking scores



Compound No.	Z	S.aureus ATCC 29213	S. aureus ATCC 33591	E. faecalis ATCC 29212	E. faecium ATTC 700221	Docking Score
69	-NHCOCH$_3$	8	4	2	2	-7.58
70	-NHCOOCH$_3$	8	4	4	4	-7.84
71		4	4	4	2	-7.46

interaction with G2618. Thus, cyclic phosphoric ester group played a pivotal role in addition to oxazolidinone ring in the pharmacodynamics of the ligand in the active site, as evident from additional interaction seen in the docking study.[22] Also, compound **47** showed best docking score compared to all the synthesized compounds (Tables 1-3, last column) and interestingly, was the most active compound among all the synthesized compounds.

Figure 2: a) overlapping of linezolid (yellow) and Compound **47** (red), b) 3D interaction diagram for Compound **47** in the binding pocket of ribosome. Black dots represent the H-bonding and the distances are in Å (Preference of color: online only)

Conclusion:

In conclusion, we have been able to identify a novel class of antibacterial agents, phenyl oxazolidinones, with special phosphorous substitution. To the best of our knowledge, this is the first report of phosphorous substituted phenyloxazolidinones showing excellent antibacterial activities. The diversity of functionality, tolerated on phosphorous atom, was remarkable. Potent activities were exhibited by most of the target molecules against Gram-positive sensitive as well as resistant strains. Compound **47**, having an 8-fold better activity in LRSA strain compared to linezolid, displayed otstanding PK profile and has been shortlisted for further safety evaluation. Docking studies also reveal additional interactions between these novel

Table 4:In vitro (MIC, µg/ml) activity of selected compounds against LRSA strain of ATCC 25923

Compound No.	LRSA strain of ATCC 25923	Compound No.	LRSA strain of ATCC 25923
27	16	**52**	16
29	16	**54**	16
35	16	**56**	16
39	16	**58**	8
40	8	**60**	8
41	8	**66**	8
47	8	**67**	16
49	16	**1** (Linezolid)	64

Table 5: Pharmacokinetic (pk) parameters of selected compounds.

Compound No.	$AUC_{(0-t)}$ (mg.h/ml)	$T_{1/2}$ (h)	C_{max} (mg/ml)	T_{max} (h)
22	68.58	1	15.01	0.25
27	33.08	2.49	7.44	1
29	114.26	0.91	27.86	0.25
39	18.97	0.68	9.032	0.25
41	33.31	1.5	10.98	0.25
47	67.67	4.2	13.65	1
51	48.36	3	17.94	0.25
54	56.20	3.1	19.6	0.5
58	14.13	1.45	6.65	0.5

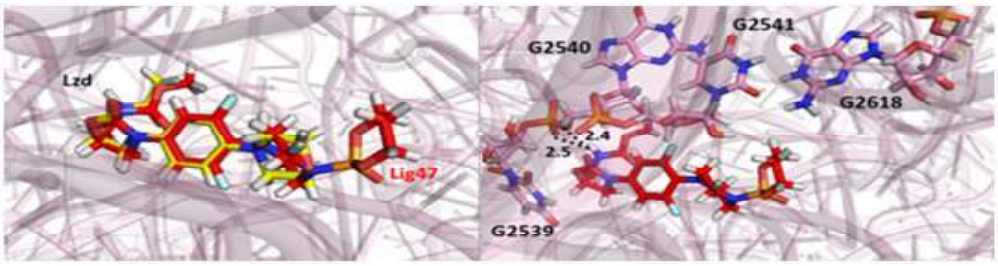


Figure 2: a) overlapping of linezolid (yellow) and Compound **47** (red), b) 3D interaction diagram for Compound **47** in the binding pocket of ribosome. Black dots represent the

phosphorous compounds and the ribosome, compared to linezolid.

Acknowledgements: The authors thank Bioinformatics Centre of NIPER, financially supported by the Department of Biotechnology (DBT), New Delhi.

Tribute - We are deeply saddened by the untimely demise of Dr. Sandeep Kanwar (an author of this article), a sincere and budding medicinal chemist, last year.

References

1. Service, R.F. Science, 270, 724(1995).
2. Swartz, M.N. Proc. Natl. Acad. Sci. U.S.A.91, 2420 (1994).
3. Tomasz, A. A. N. Engl. J. Med.330, 1247 (1994).
4. Hiramatsu, K.; Hanaki, H.; Ino, T.; Yabuta, K.; Oguri, T.; Tenover, F. C. J. Antimicrob. Chemother. 40, 135 (1997).
5. Waldvogel, F. N. Engl. J. Med.340, 556 (1999).
6. Bricker, S. J. Curr. Pharm. Des.2, 175 (1996).
7. Barbachyn, M. R.; Ford, C. W. Angew. Chem. Int. Ed.42, 2010 (2003).
8. Hutchinson, D. K. Curr. Topics Med. Chem.3, 1021 (2003).
9. Nilus, A. M. Curr. Opin. Invest. Drugs4, 149 (2003).
10. Shinabarger, D. Expert Opin. Invest. Drugs 8, 1195 (1999).
11. Colca, J. R.; McDonald, W. G.; Waldon, D. J.; Thomasco, L. M.; Gadwood, R. C.; Lund, E. T.; Cavey, G. S.; Mathews, W. R.; Adams, L. D.; Cecil, E. T.; Pearson, J. D.; Bock, J. H.; Mott, J. E.; Shinabarger, D. L.; Xiong, L.; MAnkin, A. S. J. Biol. Chem.278, 21972 (2003).
12. Shinabarger, D. L.; Marotti, K. R.; Murray, R. W.; Lin, A. H.; Melchoir, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. Antimicrob. Agents Chemother.41, 2132P (1997).
13. Hutchinson, D. K. Expert Opin. Ther. Patents14, 1309 (2004).
14. Michalska, K.; Karpiuk, I.; Król, M.; Tyski , S. Bioorg. Med. Chem. 2013, 21, 577 and the references cited therein.
15. Renslo, A. R.; Luehr, G. W.; Gordeev, M. F. Bioorg. Med. Chem. 14, 4227 (2006) and the references cited therein.
16. García-Olaiz, G.D., Alcántar-Zavala, E., Ochoa-Terán, A., Cabrera, A., Muñiz-Salazar, R., Montes-Ávila, J., Salazar-Medina, A.J., Alday, E., Velazquez, C., Medina-Franco, J.L. and Laniado-Laborín, R., Bioorg. Chem. 95, 103483 (2020)
17. Zhao, Q., Xin, L., Liu, Y., Liang, C., Li, J., Jian, Y., Li, H., Shi, Z., Liu, H. and Cao, W. J. Med. Chem., 64, 10557 (2021).
18. Alatorre-Barajas, J.A., Alcántar-Zavala, E., Gil-Rivas, M.G., Estrada-Zavala, E., Ochoa-Terán, A., Gochi-Ponce, Y., Montes-Ávila, J., Cabrera, A., Trujillo-Navarrete, B., Rivera-Lugo, Y.Y. and Alonso-Núñez, G. RSC Adv. 11, 28912 (2021).
19. Foti, C.; Piperno, A.;Scala, A.; Giuffrè, O. Molecules, 26,4280 (2021)
20. O'Connell, K. M. G.; Hodgkinson J. T.; Sore, H. F.; Welch, M.; Salmond, G .P. C.; Sprng, D. R. Angew. Chem. Int. Ed., 52, 10706 (2013).
21. Jain, R., Trehan, S.; Das, J.; Kanwar, S.; Magadi, S. K. WO2009116090, (2009). Novel antimicrobials.
22. Glide, version 5.5; Schrodinger, LLC: New York, (2009).

Current Research & Information on Pharmaceutical Sciences (CRIPS)
@NIPER, SAS Nagar, India

Research Article

A brief account of the use of persulfate beyond organic chemistry

Jojiya Grace George, Saima and Joydev K Laha*
Department of Pharmaceutical Technology (Process Chemistry),
National Institute of Pharmaceutical Education and Research
S.A.S. Nagar 160062, Punjab, India
E-mail: jlaha@niper.ac.in

Among various oxidants used for C–C and C–X bond formation in organic chemistry, an inorganic oxidant persulfate [mainly $K_2S_2O_8$, $Na_2S_2O_8$ and $(NH_4)_2S_2O_8$] embraced a prominent position due to its unique efficiency towards bond formation, cost-effectiveness, easy commercial availability, and environment friendly nature. Unlike several reviews available on the use of persulfates in organic synthesis, any specific account addressing the other uses of persulfates is currently unavailable. The current review focused on composing a brief account of the literature that describes various other uses of persulfates in biological and inter-disciplinary sciences followed by drug degradation.

Introduction

In classical chemistry term, we can describe oxidant as a chemical species that possesses the ability to gain one or more electrons, and subsequently oxidizes other substances present in a redox reaction. A vast array of oxidants that have been discovered with a decipherable use in these reactions include molecular oxygen, halogen compounds (hypochlorite, chlorite, perchlorate, chlorate), hexavalent chromium compounds such as chromic and dichromic acids and chromium trioxide, permanganate compounds, cerium compounds (ceric ammonium nitrate and ceric sulfate), $PhI(OAc)_2$, lead dioxide, TBHP, persulfates, etc. Among these varieties of oxidants, potassium persulfate is a rational choice as an oxidising agent with wide applications in anextensive range of laboratory experiments to industrial processes with listed a few.[1]

From the variety of per-oxygen family compounds, the peroxydisulfate ion ($S_2O_8^{2-}$) is known as the most prevailing oxidant and which can generate sulfate radical anion ($SO_4^{\bullet-}$) by following thermal, photolysis, radiolysis, transition metal catalysis, or redox decomposition path under mild conditions. Moreover, it has been already documented in literature and widely known that the sulfate radical anion ($SO_4^{\bullet-}$), which has been generated from any metal persulfate, is furthermore accountable for the strong oxidizing property of the metal persulfate. In addition, many reports already are available about the very strong one-electron oxidant nature of $SO_4^{\bullet-}$ possessing redox potential of 2.5-3.1 V.[2] Moreover, further analysis by various research groups explained its longer lifetime (~4 s) than hydroxyl radicals, largely due to their preference for electron transfer reactions. Though it is already known in literature that an electron transfer process could either be exergonic or endergonic, hitherto, the endergonic process might be slow due to the involvement of higher activation energy in the reaction process. $K_2S_2O_8$ is also known for its oxidizing capability for various metal salts and complexes, anions, nucleophilic radicals, and neutral organic compounds. In the present scenario, main focus is to use or employ various green chemistry principles, and thus use of $K_2S_2O_8$ becomes extensive matter of study. Various reports have appeared in literature leveraging the use of this oxidant in various metal-catalysed and metal-free organic transformations.[3] Moreover, this point needs to be highlighted mainly as $K_2S_2O_8$ is widely explored and extensively used persulfate, comparable to its other variants, such as $Na_2S_2O_8$ and $(NH_4)_2S_2O_8$, the reason behind this might be the good solubility of potassium salt in organic solvent, which further act as an adding point in the reaction transformation.[3] It has further utilized as an effective oxidant in both metal-catalyzed and metal-free reactions for the formation of C–C to C–X (X = N, O, S, P, B, Si, F, Br, I) bond across a broad range of substrates. Apart from employing this oxidant ($K_2S_2O_8$) in a wide array of oxidative organic transformations, recently the progress is seen for this oxidant to use in the field of biology and medical science. Being cheaper and readily availability, $K_2S_2O_8$ is used in a wide cluster of advanced

Keywords: Persulfates; oxidants; agriculture; disinfectant

oxidation processes (AOPs).[4] The current review is a brief account of the literature that describes various other uses of persulfates in medicinal chemistry, biological and inter-disciplinary sciences.

In 2018, a comprehensive review highlighting the recent advancement of potassium persulfate in oxidative organic transformations involving both metal catalyzed and metal-free reactions, which was contributed by our research group.[3] However, no review is available, which is specifically focused on the various roles of potassium persulfate in the research area other than organic reactions transformation. We believe that this review should fill in as a valuable reference to the scientists in creating noteworthy progress in this research area. We herein listed some of the uses of potassium persulfate in various research area other than organic synthesis.

Use of potassium persulfate in medical sciences

We are well aware about the blood transfusion, is a lifesaving procedure in which blood or blood products are transferred into patient's circulatory system. This intervention is used to tackle the emergencies and life threatening injuries. More importantly the donated blood cannot be considered as safe due to their inherent side effects.[5] However, various sensitive methods are available for the frequent detection of the blood bornediseases existing in donor's blood, risk of exposure to blood borne pathogens like AIDS, HIV could happen in allergenic blood transfusion.[6] Moreover, other complications associated with blood transfusion include agglutination caused due to inaccurate cross matching of different blood classifications, absence of accessibility of donor in specific circumstances, carefully refrigerated surrounding for blood storage and so forth.[7] To combat all these challenges, these limitations can be overcome with the utilization of artificial red blood substitutes.

Thus in this context, in 2015, RuifenXu and co-workers used potassium persulfate for an vital application in the preparation of a novel artificial red blood substitute known as grafted starch-encapsulated haemoglobin. The strategy involved in the preparation of artificial red blood cells, is to encapsulate haemoglobin with long chain fatty acid grafted with potato starch. Now the perception here is the role of potassium persulfate, which is employed as a catalyst for the purpose of grafting starch with fatty acid in DMSO, followed by the encapsulation of haemoglobin onto the grafted starch polymer that in turn resulted in the formation of grafted starch-encapsulated haemoglobin.[8]

Role of potassium persulfate as a disinfectant against microorganisms

Potassium persulfate is considered as new and efficient disinfectant, which is preferably used in livestock, aquaculture and poultry. This oxidant ($K_2S_2O_8$) exhibited higher capacity for removal of aquatic toxic material and has high efficiency in killing microorganisms. It might be possible that the potassium persulfate acts on microorganisms by oxidation, as persulfate is capable of producing $SO_4^{\bullet-}$ as the strong oxidizing agent. The main advantage associated with potassium persulfate is that it didn't affect or damage the human beings, animals and ecological environment, which further broadens the scope of persulfate application comparable to the other classical disinfectants.[9]

In another instance, use of potassium persulfate as an incredible disinfectant against antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) has been explored by Justins and co-workers. An investigation was conducted for improved ARB and ARG removal by comparing various currently used disinfection techniques like chlorination, E-beam, and prospective ozonization techniques involving potassium persulfate in combination with several catalysts. From the experiment, it was concluded that hydrogen peroxide could possibly be substituted with potassium persulfate, as it increased the effectiveness of ozone in disinfecting ARB and ARGs.[10]

Similarly, Janne Lunde and co-workers in 2013 found the application in food industries wherein they demonstrated the use of potassium persulfate as a potent disinfectant for the elimination of *L. monocytogenes* in food processing plants, which otherwise appears to persist despite regular disinfection causing prolonged contamination. It's persistence might be due to the adaptivity of the *L. monocytogenes* for all the disinfectants. The only disinfectant that *L. monocytogenes* did not adapt to was potassium persulfate and the MIC for all the strains was found to be 2500µg/ml.[11]

In 2016, Afonyushkin and co-workers found the application of potassium persulfate against some pathogenic bacteria. They studied the effect of a biocidal product, Ecocid based on potassium persulfate, on the genetic material of pathogenic bacteria specific to meat processing industry. It has been inferred from the results that the concentration of the chromosomal DNA of *Clostridium* perfringes was reduced to 28-29 times due to the tested disinfectant based on potassium persulfate.[12]

In other report, which also documented the application of potassium persulfate for bactericidal activity was given by Ashley and co-workers wherein

they contemplated the specific bactericidal action of potassium persulfate, which demonstrated that potassium persulfate behaves as a "physicochemical" germicide preferentially attacking *B. fluorescens non-liquefaciens*. This bactericidal activity of potassium salts might be because of the way that these salts induce physical changes in the proteins as well as exerts their oxidising capacities on the microorganisms.[13]

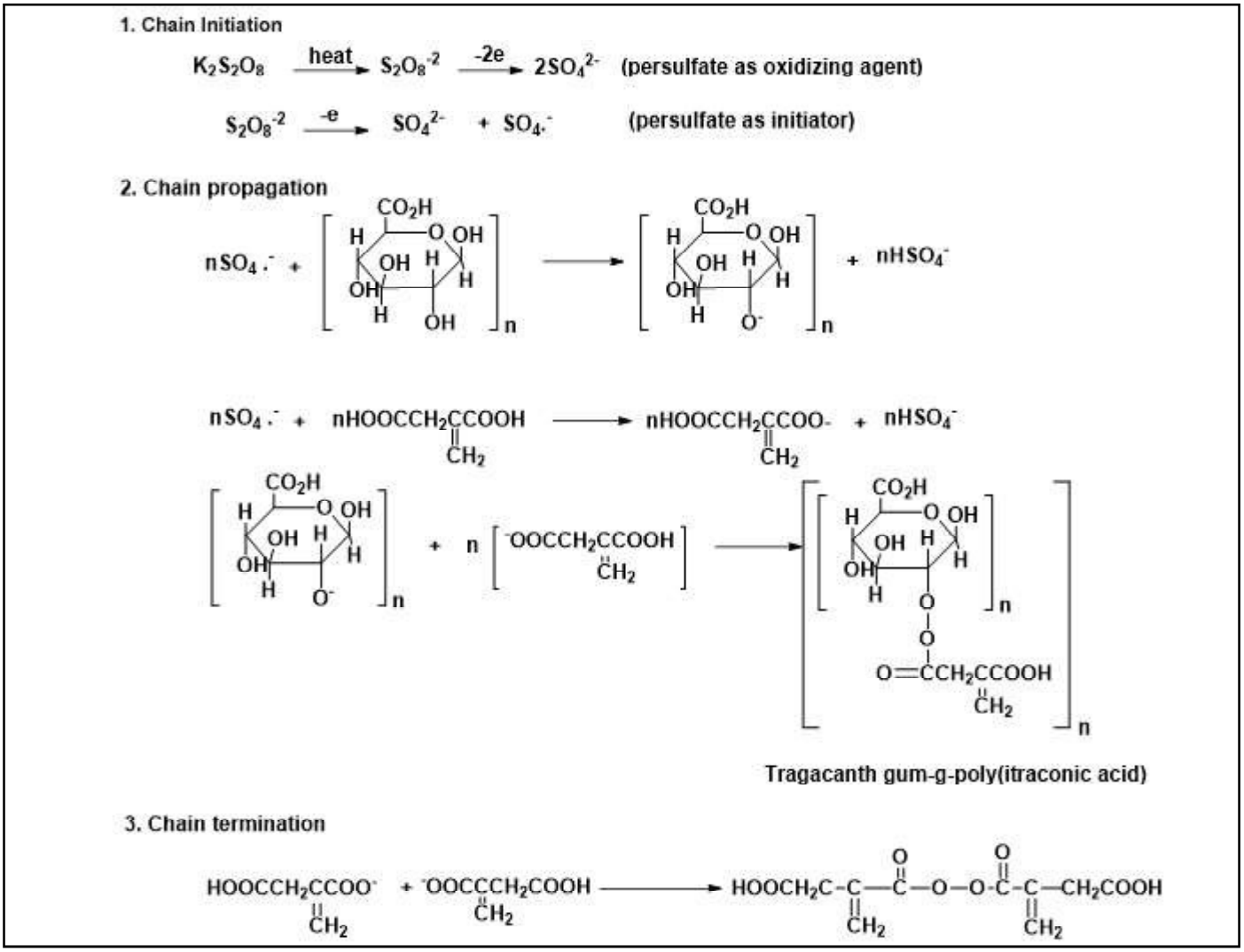
Potassium persulfate as an initiator in polymer chemistry and nanotechnology:

Potassium persulfate being a strong oxidant, has been very nicely employed used to initiate polymerization reactions, which can indeed found application in the polymer making industries for the synthesis of important polymers, beneficial in medicinal chemistry, or for commercial use.

Thus in 2018, Deepak and co-workers used potassium persulfate for grafting wherein potassium persulfate acts as an initiator for grafting of tragacanth gum with itaconic acid in the presence of N,N'-methylene-bis-acrylamide as cross linker. In addition, the synthesised polymer has significant antimicrobial efficacy against E.Coliby agarwell diffusion assay.

Scheme 1nicely demonstrated the mechanism of nanohydrogel formation. The major steps involved in polysaccharide modification, involve the formation of ester and ether linkage with sugar hydroxyl groups. These hydroxyl groups make the reaction site assessable for alterations and cross linkages in tragacanth gum.[14]

Adding another example for polymer synthesis application, in 2017, Sood and co-workers utilized potassium persulfate as a cross linking agent. The group has demonstrated the use of microwave for the preparation of carboxymethyl cellulose-cl-poly (lacti acid-co-itaconic acid) hydrogel in the presenc of potassium persulfate as linking agent. During this preparation process, N,N'-methylene-bis-acrylamide serves as an initiator. The effect of potassium persulfate was studied, which showed that maximum grafting could be obtained at 0.3 g of potassium persulfate. Active sites are generated on the polymeric backbone by the initiator and monomers causing increase in grafting.[15]



Scheme 1. Mechanism of grafting itraconic acid on tragacanth gum.

Another hydrogels formation was demonstared by Guanghua et.al, wherein they prepared super absorbent hydrogels by grafting N-succinyl chitosan with poly (acrylic acid-co-acrylamide). In the preparation process, potassium persulfate and N,N'-methylenebisacrylamide were used as initiator andcross linker, respectively. In the study, the effect of potassium persulfate content on the water absorbency for the super absorbent hydrogel was observed, which showed that there was an increase in the water absorption capacity of hydrogel from 767 to 1375g/g and then decreased to 503g/g. The absorption of PAMAM dendrimer in alkaline or lower concentration solution also inhibits the growth of Escherichia coli, which demonstrates its antibacterial application.[16]

To extend further application of potassium persulfate in the recent technology, various reports are available in literature in the nanoscience wherein its utilization has been employed. Thus, in 2017, Dipankar and co-workers used potassium persulfate as an initiator for the preparation of functionalized dextrin based cross-linked, pH responsive and biocompatible nanogel for the targeted delivery of doxorubicin hydrochloride to human osteocarcoma cancer cell lines. In this report, they used Michael-type addition reaction for the preparation of nanogel using dextrin. The other components involved in the reaction include N,N'-methylene bisacrylamide that functions as a cross linker and acrylic acid that serves as a monomer.[17]

Guanghua et al. synthesized quaternary ammonium chitosan-g-poly(acrylic acid-co-acrylamide) superabsorbent hydrogels from acrylic acid, acrylamide and quaternary ammonium chitosan, wherein potassium persulfate was used as an initiator and N,N' methylenebisacrylamide as a cross linker, respectively. The effect of potassium persulfate content on the water absorbency of the super absorbent hydrogel was evaluated during the study. They also observed that with a change in the potassium persulfate content from 0.25 to 0.75 %, an increase in water absorbency in distilled water from 292 to 429 g/g was observed and reached the maximum with 0.75 % of the initiator content. Further increase in the initiator content leads to lowering of water absorbency. The antibacterial study indicated that the antibacterial activity was improved on increase in the content of initiator, but the activity would decrease if more initiator is added beyond a certain limit.[18]

In polymer science, another example was given by Rajeev and co-workers in 2016, where they used potassium persulfate as a thermal initiator for the free radical polymerization initiation during the synthesis of poly (acrylamide)-based hydrogel, which serves as the polymer matrix in the preparation of Gum rosin acrylamide copolymer-based nanogel. Formation of GrA-cl-poly(AAm) is facilitated by grafting poly(acrylamide)chains onto the polymeric backbone employing $OH^{\bullet}$ as the most active site. Potassium persulfate is decomposed to form $SO_4^{\bullet-}$, which reacts with water to generate $OH^{\bullet}$ free radicals at an ambient temperature. The polymeric backbone reacts with the $OH^{\bullet}$ free radical and monomer resulting in the formation of active sites. Finally, the active graft polymer is formed by grafting of poly (acrylamide) chains onto this active site of backbone, which in turn undergoes crosslinking with MBA to from cross linked hydrogel having more antibacterial activity against S.aureus as compared to *E.coli*.[19]

In 2015, Bajpai and Mamta employed potassium persulfate as an initiator and N,N'-methylene bisacrylamide as cross-linker for the free radical initiated aqueous polymerization of sodium acrylate monomer for the fabrication of gum acasia/ poly(sodium acrylate)semi-interpenetrating polymer networks(Semi-IPN) that was further used for the in situ preparation of *Syzygiumaromaticum* loaded silver nanoparticles having antibacterial activity against E.coli.[20]

In 2012, Nadia and co-workers used potassium persulfate as an initiator in the grafting of carboxymethyl chitosan with N-acryloyl, N'-cyanoacetohydrazide in homogenous aqueous phase. Nitrogen radicals were reported to be formed on carboxylmethyl chitosan after reacting with potassium persulfate, which upon radical addition to N-acryloyl,N'-cyanoacetohydrazide and subsequent polymerization generated various copolymers. The maximum grafting yield achieved for 0.03 mol/L potassium persulfate was 44.8 % at 60 ºC within 2 h. These grafted polymers showed an inhibitory effect on both *E. coli* and *Staphylococcus aureus* bacteria followed by *Aspergillus favus* and Candida albicans fungi which were better than those of chitosan and carboxymethyl chitosan themselves.[21]

In the same year, Kamel prepared silver-nanoparticle (AgNP) containing paper exhibiting antibacterial activity. Polymerization was carried out by grafting acrylamide onto bagasse paper sheets under microwave conditions using potassium persulfate as an initiator. The loading of silver-nanoparticle on the acrylamide grafted baggase paper sheets occurs by in situ reduction of silver nitrate with citrate molecule as a stabilizing ligand.[22]

In 2011, Anirudhan and Sandeep synthesized and characterized a novel pH-controllable hydrogel by in situ intercalation graft copolymerization of 2-acrylamido-2-methylpropane sulfonic acid (AMPS)

with N-malleoylchitosan (MACTS) intercalated montmorillonite to form 2-acrylamido-2-methylpropane sulfonic acid (AMPS) grafted N-malleoylchitosan (MACTS) intercalated montmorillonite for anticancer drug delivery. This graft polymerization was carried out by potassium persulfate as a free radical initiator and N, N-methylenebsacrylamide as a cross-linking agent.[23]

Another example in the nano drug delivery was given in 2011 by Julio and Edward, wherein they developed a convenient method for the preparation of emulsified polyacrylate nanoparticles for the delivery and protection of ß-lactam antibiotics. In this example, potassium persulfate acts as a water-soluble initiator in the emulsion polymerization using butyl acrylate and styrene in water in the ratio 7:3, containing sodium dodecyl sulfate as a surfactant resulting in polyacrylate-based nanoparticles. These emulsions were purified by centrifugation and dialysis, which upon lyophilisation formed a homogenous dried powder, which further can be reconstructed by an aqueous diluent forming a nanoparticle emulsion for the application in the field of drug delivery.[24]

Hydrogels synthesis application for bacterial inhibition was demonstrated by Saxena and Bajpai in 2010, prepared cationic-resin (seralite SRC-120)-loaded poly (acrylamide) gels by free radical polymerization of acryl amide in the presence of resin particles. This reaction was initiated by potassium persulfate and methylene bisacrylamide serves as the cross-linker. The resin loaded hydrogel exhibited minimum swelling property and was found to have greater inhibitory activity against E.coli as compared to silver-nano particle loaded hydrogels.[25]

Huajiang et al. used potassium sulphate as an initiator for the free radical polymerization of an antibacterial quaternary ammonium acrylic monomer, which was synthesized by the quaternization of 2-dimethylamino ethyl methacrylate with dimethylsulfate. The corresponding homo-polymers when tested against E. coli and S. aureus showed significantly stronger antibacterial activity as compared to the monomer. From the study it was concluded that the number of polymer molecules were increased directly with increasing the potassium persulfate concentration, while the degree of polymerization was decreased which on the other hand indirectly lead to a decrease in the viscosity.[26]

In 2008, Loannis and co-workers found application in the emulsion formation and thus employed potassium persulfate as an initiator for the emulsion polymerization of styrene to form anionic polystyrene lattices that was used in the synthesis of hollow ceria nanospheres. Radicals are penetrated into the micelles and the molecules present in the monomer will diffuse continually from the monomer spheres to the micelles through the solvent until all the monomers are eliminated to form the polymers.[27]

In 2007, Sampath and co-workers prepared glycosylated polyacrylate nanoparticle from glycosylated drug monomers by the emulsion polymerization technique in which potassium persulfate was utilized as a radical initiator. The emulsion polymerization was carried out using a mixture of butylacrylate-styrene in 7:3 ratio (w:w) in the presence of sodium dodecyl sulfate as surfactant along with potassium persulfate which served as a radical initiator. The average diameter of the glyconano particles was about 40 nm by dynamic light scattering (DLS). The resulting nanoparticles possessed equivalent or improved in-vitro antibacterial activity as compared to their precursor monomers.[28]

Bajpai and co-workers found use of potassium persulfate in wound dressing and thus applied it as an initiator along with the cross linker, N,N'-methylene-bis acrylamide in soaking plain cotton cellulose (CC) fibres in a reaction mixture containing monomer acrylic acid to produce cotton cellulose/ polyacrylic composite fibres. These fibres could be utilized to release the entrapped drug Gentamicin sulfate in the physiological fluid for burn/wound dressing applications. These fibres when tested demonstrated significant mechanical strength and fair biocidal activity against *E. coli*.[29]

Fahreia and co-workers grafted N-acryloylmorpholine (NAM) onto chitosan by utilizing 1% acetic acid as the solvent under homogenous conditions, wherein potassium persulfate and sodium sulfite were used as the redox initiator. The grafted polymer remarkably demonstrated an improvement in crystallinity along with a stronger inhibitory activity on bacteria and fungi as compared to chitosan.[30]

In 2013, Wenning and co-workers designed potassium persulfate as an oxidant for the preparation of divalent silver oxide-diatomite (AgO-d) hybrids by chemical oxidation using silver nitrate and diatomite as the raw materials. AgO-d hybrids when tested for antibacterial activity showed excellent bactericidal activity against *S. aureus* and *E. coli.*[31]

In 2013, Fakhreia et.al employed potassium persulfate in combination with sodium sulfite to form a redox system for the graft polymerization of the novel monomer 2-(furan-2-carbonyl) acrylonitrile onto chitosan under heterogeneous conditions. The grafted copolymers significantly led to a retardation in the bacterial and fungal growth and moreover, depicted good swelling properties indicating that they can also be employed as hydrogel materials.[32]

In 2010, Dipankar and co-workers employed potassium persulfate oxidation at optimized conditions for the depolymerisation of alginic acid biopolymer to prepare low molecular weight alginic

acid based nanoparticles to serve as an effective drug delivery system for the management of drug resistant bacteria. The potassium persulfate concentration along with the pH and reaction time highly influences the alginic acid depolymerisation and hydrodynamic diameter decreases with increase in the concentration of potassium persulfate.[33]

Role of potassium persulfate in the degradation of organic contaminants and harmful chemicals

With the advancement, there is also an increase in the waste by product and pollutants in the environment. And thus increasing pollutants has surfaced a way for studying the use of potassium persulfate in improving treatment process against these hazardous chemicals. Moreover, recent waste water treatment processes rely on persulfate mediated advanced oxidation process (AOP) in order to degrade a wide range of organic pollutants and contaminants. An input of energy in the form of heat or photon leads to peroxide and activation in waste water treatment. Additionally, direct electrolysis or reduced metals, metal oxides are often adopted approaches that initiate peroxide bond breaking redox reaction. The basic principle behind these processes involves the production of $SO_4^{\bullet-}$ that initiates a cascade of reactions. This, in turn, leads to the formation of intermediate oxidants such as H_2O_2, $HSO_5^{\bullet-}$ and other reactive hydroxyl radicals, which could lead to the degradation of organic contaminants and harmful chemicals.[34]

Metformin, an anti-diabetic drug, is one of the pharmaceutical contaminant that can be found in local and clinic waste water, which are associated to influence the offset with biological systems and the human wellbeing. In 2018, Sawsen and Nadia used potassium persulfate as an electron acceptor in addition to hydrogen peroxide and sodium persulfate to improve the TiO_2 photo catalytic activity to investigate the degradation of metformin in aqueous medium under sunlight irradiation. Potassium persulfate was used as an additive in order to supplement the dissolved oxygen that would in turn lead to reaction rate improvement in the photo degradation by reacting with the electrons of the conduction band to form the intermediate radicals. In the process, the effect of different concentrations of potassium persulfate was investigated. The study showed that the degradation rate of metformin was increased at high potassium ion concentrations and achieved 91.6 % after 210 min of sunlight irradiation.[35]

In 2012, Subramanian and co-workers utilized potassium persulfate for degradation of Malachite green due to its genotoxic and carcinogenic properties. Malachite green was employed as a disinfectant, as a biocide in aquaculture, as an antihelminthic and also as a dye in paper and textile industries. Microbial assay revealed that the treatment of malachite green by potassium persulfate in presence of complex led to the removal of antibacterial activity indicating that this oxidation system has the ability to decrease the toxicity of malachite green towards the bacteria.[36]

In 2011, Saien and co-workers used potassium persulfate to facilitate the degradation of Triton X-100, most famous anionic surfactant that forms a significant fraction of dissolved organic pollutants in water ecosystems. It possesses wide practical applications in almost every type of liquid, paste and powdered cleaning compounds, ranging from heavy-duty industrial and agrochemical products to gentle detergents. 80.9 % degradation of TX-100 in 60 min and a complete degradation in only 30 min is achieved by applying homogenous AOPs, UV/H_2O_2 and UV/KPS under the optimum conditions of pH and temperature in addition to 270.3 mg/L of potassium persulfate.[37]

Summary and outlook

Potassium persulfate is widely used as a powerful oxidant in various organic transformations. Although metal persulfate seems to be a proprietary reagent for organic chemists, its application in areas other than organic chemistry has also been the subject of intensive investigation. This review highlighted the possible contributions of potassium persulfate in the field of biology and medical field by playing an important role for using as disinfection in biomedical field by degradation of contaminants and harmful chemicals. Further researches are needed in studying the utilization of metal persulfate that can contribute effectively in more spheres of environment and life as in the present scenario, The waste water treatment is on high upsurge, so we can use the potassium persulfate for the treatment of the water and in nano drug delivery system also.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the NIPER S.A.S. Nagar.

References

1. Minisci F, Citterio A and Giordano C 1983 Electron-transfer processes: peroxydisulfate, a useful and versatile reagent in organic chemistry. Acc. Chem. Res.16 27.
2. Matzek L W and Carter K E 2016 Activated persulfate for organic chemical degradation: A review Chemosphere 151 178.
3. Mandal S, Bera T, Dubey G, Saha J and Laha J K 2018 Uses of $K_2S_2O_8$ in Metal-Catalyzed and Metal-Free Oxidative Transformations. ACS Catal.8 5085.
4. Pari S, WangI A, Liu H and Wong B M 2017Sulfate radical

oxidation of aromatic contaminants: a detailed assessment of density functional theory and high-level quantum chemical methods. Environ. Sci. Processes Impacts19 395.
5. Sahu S, Hemlata and Verma A 2014 Adverse events related to blood transfusion. Indian J. Anaesth. 58 543.
6. Kleinman S, Lelie N and Busch M P 2009 Infectivity of human immunodeficiency virus-1, hepatitis C virus and hepatitis B virus and risk of transmission by transfusion.Transfusion 49 2454.
7. Rudowski W J 1971 Complications associated with blood transfusion. In Progress in surgery Karger Publishers, 978.
8. Xu R, Zhang J, Zhou P, Yang R, Feng X and Xu L 2015 A novel artificial red blood cell substitute: grafted starch-encapsulated hemoglobin. RSC Adv. 5 43845.
9. CN101785480A water purifying and sterilizing disinfectant.
10. Oh J, Salcedo D E, Medriano C A and Kim S 2014 Fate of Antibiotic Resistant Bacteria and Genes during Wastewater Chlorination: Implication for Antibiotic Resistance Control. J. Environ. Sci. 26 1238.
11. Lundén J, Autio T, Markkula A, Hellström S and Korkeala H 2003 The Connection between Persistent, Disinfectant-Resistant Listeria monocytogenes Strains from Two Geographically Separate Iberian Pork Processing Plants: Evidence from Comparative Genome Analysis. Int. J. Food Microbiol. 82 265.
12. Afonyushkin V N, Tabanyukhov K A, Cherepushkina V S, Khomenko Y S and Tatarchuk O P 2016 Effect of disinfactants based on potassium persulfate hydrogen peroxide, glutaraldehyde and quartenary ammonium compounds on the genetic material of pathogens bacteria specific to meat industry. Theory and practice of meat processing 1 54.
13. Cooper E A and Mason J 1927 Urinary catheter-associated infections Epidemiol. Infect. 26 118.
14. Pathania D, Verma C, Negi P, TyagiI, Asif M, Kumar N S and Gupta V K 2018 Novel nanohydrogel based on itaconic acid grafted tragacanth gum for controlled release of ampicillin. Carbohydr. Polym.196 262.
15. Sood S, Gupta V K, Agarwal S, Dev K and Pathania D 2017 Studies on anticancerious and photocatalytic activity of carboxymethyl cellulose-cl-poly(lactic acid-co-itaconic acid)/ZnO-Ag nanocomposite Int. J. Biol. Macromol. 101 612.
16. He G, Ke W, Liu L, Li Y, Zheng H, Yin Y and Yi Y 2017 Preparation, swelling and antibacterial behaviors of N-succinyl chitosan-g-poly(acrylic acid-co-acrylamide) superabsorbent hydrogels. Journal of Wuhan University of Technology-Mater. Sci. Ed. 32 963.
17. Das D, Rameshbabu A P, Ghosh P, Patra P, Dhara S and Pal S 2017Biocompatible nanogel derived from functionalized dextrin for targeted delivery of doxorubicin hydrochloride to MG 63 cancer cells. Carbohydr. Polym. 171 27.
18. He G, Ke W, Chen X, Kong Y, Zheng H, Yin Y and Cai W 2017Preparation and properties of quaternary ammonium chitosan-g-poly(acrylic acid-co-acrylamide) superabsorbent hydrogels. React. Funct. Polym.111 14.
19. Jindal R, Sharma R, Maiti M, Kaur A, Sharma P, Mishra V, Jana A K 2016 Synthesis and Characterization of Novel Reduced Gum Rosin-Acrylamide Copolymer-Based Nanogel and Their Investigation for Antibacterial Activity. Polym. Bull. 74 2995.
20. Bajpai S K and Kumari M A2015 Investigation of curcumin release from chitosan/cellulose micro crystals (CMC) antimicrobial films. Int. J. Biol. Macromol. 80 177.
21. Mohamed N A and Abd El-Ghany N A 2012 Synthesis, Characterization, and Antimicrobial Activity of Carboxymethyl Chitosan-Graft-Poly(N-acryloyl,N'-cyanoacetohydrazide) Copolymers. J. Carbohydr. Chem. 31 220.
22. Kamel S 2012 Rapid synthesis of antimicrobial paper under microwave irradiation. Carbohydr. Polym. 90 1538.
23. Anirudhan T S and Sandeep S 2011Synthesis and characterization of a novel pH-controllable composite hydrogel for anticancer drug delivery. New J. Chem.35 2869.
24. Garay-Jimenez J C and Turos E A 2011 Physical properties and biological activity of poly(butyl acrylate–styrene) nanoparticle emulsions prepared with conventional and polymerizable surfactants. Bioorg. Med. Chem. Lett. 21 4589.
25. Saxena S and Bajpai S K 2010 Is a cationic-resin-loaded polymericgel a better antibacterial material than a nanosilver-loadedgel? D Des. Monomers Polym. 13 157.
26. Zuo H, Wu D and Fu R 2010 Synthesis of antibacterial polymers from 2-dimethylamino ethyl methacrylate quaternized by dimethyl sulfate. Polym. J. 42 766.
27. KartsonakisI A, Liatsi P, Daniilidis I and Kordas G 2008 Encapsulation of the Corrosion Inhibitor 8-Hydroxyquinoline into Ceria Nanocontainers. J. Am. Ceram. Soc. 91 372.
28. Abeylath S C, Turos E, Dickey S and Lim D V 2008 Glyconanobiotics: Novel carbohydrated nanoparticle antibiotics for MRSA and Bacillus anthracis. Bioorg. Med. Chem. 16 2412.
29. Bajpai S K, Das P and Sharma L 2013 In situ formation of silver nanoparticles in poly(N-isopropyl acrylamide) hydrogel for antibacterial applications. J. Macromol. Sci. A. 50 55.
30. Al-Sagheer F A, Ibrahim E I and KhalilK D 2014 Crystallinity, antimi-crobial activity and dyeing properties of chitosan-g-poly(N-acryloyl morpholine) copolymer. Eur. Polym. J.58 164.
31. Sood S, Gupta V K, Agarwal S, Dev K and Pathania D 2017 Controlled release of antibiotic amoxicillin drug using carboxymethyl cellulose-cl-poly(lactic acid-co-itaconic acid) hydrogel. Int. J. Biol. Macromol.101 612.
32. Sagheer F A Al, Khalil K D and Ibrahim E I 2013 Synthesis and characterization of chitosan-g-poly(2-(furan-2-carbonyl)-acrylonitrile): Grafting of chitosan using a novel monomer prepared by a BaylisHillman reaction. Eur. Polym. J. 49 1662.
33. Ghosh D, Pramanik A, Sikdar N and Pramanik P, 2011 Synthesis of low molecular weight alginic acid nanoparticles through persulfate treatment as effective drug delivery system to manage drug resistant bacteria. Biotechnol. Bioproc. E. 16 383.
34. Lee J, von Gunten U and Kim J H 2020 Persulfate-Based Advanced Oxidation: Critical Assessment of Opportunities and Roadblocks. Environ. Sci. 54 3064.
35. Gokulakrishnan S, Parakh P and Prakash H 2012 Photodegradation of methyl orange and photoinactivation of bacteria by visible light activation of persulphate using a tris(2,2'-bipyridyl)ruthenium(II) complex. J. Hazard. Mater. 213 19.
36. Saien J, Ojaghloo Z, Soleymani A R and Rasoulifard H M 2011Photocatalytic decomposition of direct red 16 and kinetics analysis in a conic body packed bed reactor with nanostructure titania coated Raschig rings. Chem. Eng. J., 167, 172.
37. Nezar S and Laoufi N A 2018 Electron acceptors effect on photocatalytic degradation of metformin under sunlight irradiation. Sol. Energy164 267.

Research Article

Drug Metabolism: Pharmacoinformatics Efforts

Kanika Manchanda and Prasad V. Bharatam*

Department of Medicinal Chemistry and Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar - 160 062, Punjab, India

Cytochromes P450 are oxidizing enzymes, and among many subfamilies of cytochromes, a few play a significant role in drug metabolism. When a drug undergoes metabolism, cytochromes carry out many biochemical transformations like (i) conversion of prodrug to a drug, (ii) conversion into polar metabolites for easy excretion, or (iii) production of those metabolites which may be reactive and cause toxic effects in the body. Various experimental methods, such as mass spectral analysis, NMR, and in vitro analysis, can be utilized to determine the mechanisms involved in generating reactive metabolites that may cause toxicity. Pharmacoinformatic methods provide detailed atomic-level information, which is challenging to acquire from experimental studies, and this complementary information is essential in modern-day science. Methods like molecular dynamics, molecular docking, quantum chemical methods, and AI methods have become available in pharmacoinformatics to do in silico studies. This review aims to explore the use of quantum chemical studies and chemoinformatics, as well as the application of artificial intelligence, in investigating drug metabolism.

1. Introduction to drug metabolism

Drug metabolism is a pharmacokinetic process that transforms hydrophobic regions of drugs into hydrophilic regions through biotransformation.[1] The purpose of drug metabolism is to promote the drug's effectiveness in the body and ensure safe elimination from the body.[2] This involves the enzymatic breakdown of drugs or xenobiotics, such as by the CYP450 family of enzymes. This process is essential because the fat-soluble nature of drugs can prolong their stay in the body and cause unwanted side effects.

Drug metabolism can be classified into two main reactions: phase I and II. Phase I reactions involve modifying the drugs through oxidation, reduction, and hydrolysis, making it easier to eliminate from the body. However, these metabolized drugs may not be readily excreted from the body. Drugs require further modifications, which take place during phase II metabolism involving conjugation reactions.[3] In some instances, prior to undergoing phase II reactions, the drug metabolism process can generate reactive metabolites that may cause harm or toxicity.

Various factors can affect drug metabolism.[4] At the molecular level, the 3D structure of a drug and electronic structure play a crucial role. The active site of cytochrome enzymes, including their size, shape, exit channel and entry channel and amino acid composition, determines. The physiological and pathological conditions, diet, and environment influence the path and rate of biotransformation. Often, different organisms influence these biochemical reactions differently.[5]

Reactive metabolites (RMs) are produced due to drug metabolism and are highly electrophilic.[6] They can interact with biomolecules such as proteins, lipids, and nucleic acids, ultimately leading to cellular dysfunction. They can form covalent bonds with macromolecules, causing oxidative stress, protein malfunction, DNA damage, and cell death.[7]

The intricate details of the biotransformation happening in the body can be easily known using the in silico tools. These methods help us understand chemical interactions at the atomic scale, surface properties, reaction coordinate diagram of the biotransformation, hydrophobicity vs hydrophilicity balance,[8] the complete set of three-dimensional structures depicting the pathways of the reaction, charge distribution at various atoms of the drugs, are corresponding to the results emerging from experimental methods. Several in silico predictive tools became available that employ the physiochemical descriptors of drugs. Artificial intelligence (AI) methods are also becoming practical in evaluating drug metabolism and associated

toxicity. A few details are presented in this review.

2. Studies utilizing quantum chemistry to investigate drug metabolism and toxicity

Quantum chemistry involves use of Schrodinger wave equation to solve for the wavefunction of every electron in a chemical substance, including drugs and their metabolites. Various methods can be used to perform quantum chemical calculations. The method depends upon the type of system (small or large molecules). The most commonly used method for modelling CYP450-catalyzed metabolic reaction is density functional theory (DFT). B3LYP hybrid functional is the widely used density functional in which Hartree-Fock exact exchange[9] and Lee-Yang-Parr correlation functional[9] are mixed. The most realistic and economical approach for obtaining atomistic details of the enzyme catalytic environment is achieved using the two components: quantum mechanics and molecular mechanics (QM/MM) method, as the pure quantum chemicals method, take lots of computational time.[10]

Two case studies in this review are discussed in which the quantum chemical techniques were used. The results gave atomistic details of all the drugs and their metabolites. Energy values found using QC calculations were further used to determine the potential energy surface. In contrast, HOMO and LUMO values and shapes were used to predict the toxicity of the drugs and their metabolites.

2.1 Mechanistic details of metabolic conversion of remdesivir

Remdesivir (GS-5734) is an antiviral nucleoside analogue that showed positive findings against the Ebola virus. It was developed by Gilead Sciences in 2015.[11, 12] US FDA approved this drug for emergency use for potential COVID-19 treatments.[12] Despite initial hopes for its efficacy, the results of a randomized clinical trial indicated that remdesivir is not effective enough in reducing the mortality rate of hospitalized patients.[13]

Mackman et al. synthesized remdesivir that metabolized into active triphosphate inside the body.[14] Gotte et al. (2020) reported on the termination of RNA synthesis that inhibits the coronavirus replication cycle with the help of enzyme kinetic studies. The active triphosphate form of remdesivir effectively inhibits the replication cycle of the coronavirus by incorporating it into the virus's RdRp (nsp12) enzyme.[15] The old literature studies hinted at the metabolic activation pathway of remdesivir. Several enzymes were involved in the mechanism for protides proposed by McGuigan et al., which was based on experimental studies examining the metabolic conversion of 2′,3′-didehydro-2′,3′-dideoxythymidine monophosphate (d4T-MP) to its active metabolite (d4T-TP).[16] Furman et al. proposed a similar biotransformation pathway for PSI-7851, a phosphoramidate prodrug of 2′-deoxy-2′-α-fluoro-α-C-methyluridine-5′-monophosphate and its diastereomer PSI-7977.[16] Warren et al. performed *in vitro* metabolism studies of remdesivir. The concentrations inside the cell of various metabolites at various periods were also reported.[11]

Computational studies have also been performed to explore the drug's action. According to Zhang et al., the binding affinity of the triphosphate metabolite of remdesivir to SARS-CoV-2 RdRp was higher than that of ATP.[17] Jung et al. also investigated the binding site of remdesivir, and its monophosphate metabolite (GS-441524) on several non-structural proteins of SARS-CoV-2.[18] Wang and co-workers studied the mechanism of inhibition of SARS-CoV-2 RdRp.[19]

Our group utilized quantum chemical analysis to obtain atomic-level information about remdesivir metabolism.[20] Since the mass spectroscopy reports provided information on metabolites but no information on the three-dimensional (3D) conformations of the metabolites and the intermediates, the quantum chemical methods were employed to explore the drug metabolism mechanisms with 3D structures of each. A model system of remdesivir (MSR, MeOC(O)C(Me)NHP(O)(OMe)OPh) was built to study the biotransformation pathway of ProTide type prodrugs involving a pentacoordinate phosphorus intermediate, a cyclic anhydride intermediate and various P-N and P-O bond hydrolysis steps.

DFT calculations using the Gaussian09 software package were employed in the study. All the 3D structure optimizations were performed using hybrid functionals with Becke 3 Lee Yang Par (B3LYP) method. The X-Ray diffraction structure of remdesivir (CCDC No. 1525840) has been employed as an initial coordinate to maintain similar 3D geometry between MSR and remdesivir. The saddle points of the structures were established using the analytical frequencies estimated for the optimized structures. These computational studies gave an insight into the complete biotransformation pathway.

The critical function of phospharamidase enzyme in P-N bond hydrolysis of alanine metabolite of remdesivir (**R_M2**) was reported through the utilization of a combined approach involving molecular docking and quantum mechanics. The reaction involved the conversion of remdesivir into a

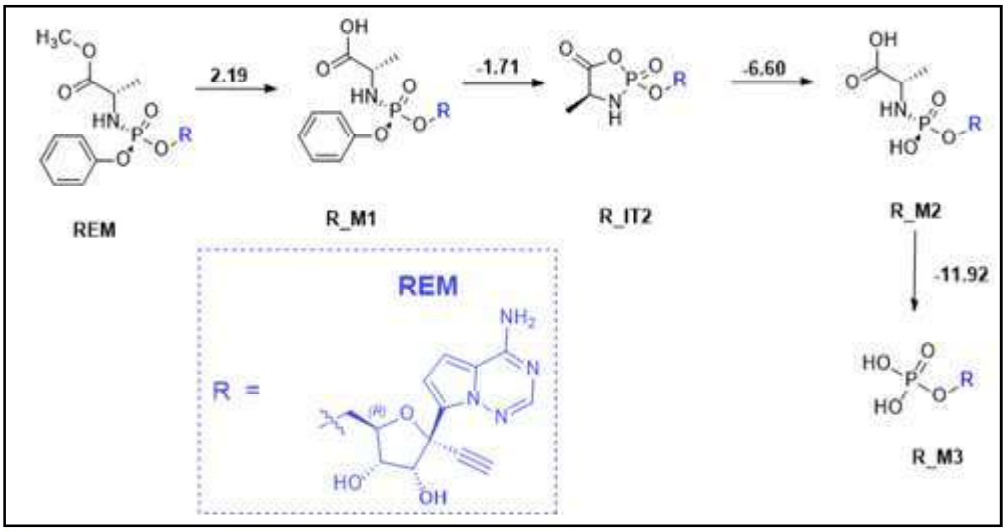


Figure 1. Metabolic pathway of remdesivir. Relative energy values (in kcal/mol) on the arrow marks represent ΔG values.

monophosphate derivative via four steps, i.e., hydrolysis of the ester bond to give free carboxylic acid metabolite followed by the intramolecular reaction of the carboxylic group at the phosphate centre and removal of phenol to yield **R_IT2** to give alanine metabolite **R_M2**. Further, the P-N bond hydrolysis of **R_M2** gave the **R_M3** metabolite (Figure 1). The formation of monophosphate metabolite of remdesivir **R_M3** through P-N bond hydrolysis in phosphoramidate metabolite **R_M2** required an energy of 41.78 kcal/mol which indicated the importance of an enzyme in P-N bond cleavage since this energy barrier is very high. The nucleoside phosphoramidase enzyme, human histidine triad nucleotide-binding protein 1 (hHint1), is responsible for cleaving the P-N bond in ProTide prodrugs. The calculations and analysis showed that the barrier was reduced by 27.52 kcal/mol. This analysis indicated the indispensable role of an enzyme in the P-N bond cleavage of phosphoramidate metabolite of remdesivir **R_M2**.

2.2 Biotransformation and toxicity prediction of drugs containing thiazole ring using quantum analysis

The biotransformation of drugs by cytochrome P450s (CYPs) can form electrophilic reactive metabolites (RMs), which can covalently bind with essential cellular macromolecules. The reactive metabolites can disrupt biological functions, leading to drug-drug interactions or idiosyncratic adverse drug interactions.[21,22,23] Reactive metabolites (RMs) are known to be generated by drugs that contain thiazole and aminothiazole groups. Several drugs containing thiazole rings, including sudoxicam, thiabendazole, and ritonavir, have been reported to be toxic. Sudoxicam, for example, is transformed through epoxidation, followed by a ring-opening reaction, which results in the formation of thioamides that can form covalent adducts.[22,24,25] The reactive metabolites from thiazole and thiabenzadole formed in mice were reported by Mizutani et al.[26,27] In their study, Kalgutkar and colleagues demonstrated that a 2-amino-4-aryl-thiazole functional group could undergo bioactivation in human liver microsomes. They also characterized the resulting GSH adducts using LC-MS/MS and NMR techniques.[28] Subramanian and colleagues also studied the cytochrome-mediated epoxidation of AKT inhibitors based on 2-aminothiazole.[29] Our group focussed on obtaining molecular details and explored the reaction coordinate diagram of drugs containing thiazole and aminothiazole rings using the model systems.[30]

Subramanian et al. utilized molecular docking studies with the Glide software in the Schrodinger suite of programs to investigate the site of metabolism for drugs containing thiazole.[31] They visually examined the highest-ranked poses and analyzed their interactions with active site residues and the heme Fe=O centre. Additionally, the authors used DFT (B3LYP(SCRF)/6-311++G(d,p)//6-31+G(d)) through the Gaussian 09 suite of programs to study the mechanisms associated with four crucial biotransformation pathways (epoxidation, S-oxidation, N-oxidation, and oxaziridine formation) of the model compounds thiazole (TZ) and aminothiazole (ATZ).

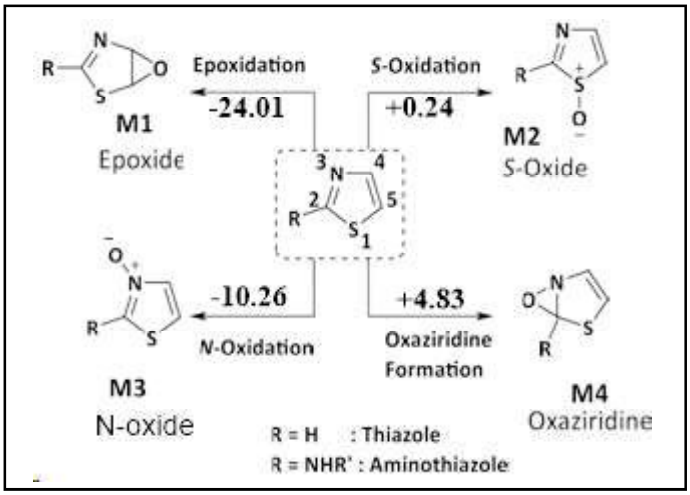


Figure 2. Biotransformation pathways of drugs containing thiazole rings include ΔG values, expressed in units of kcal/mol, indicated on the arrow as relative energy values.

The epoxidation reaction was found to be more favourable as this reaction is exergonic by 24.01 kcal/mol and requires a small energy barrier. The process begins with the attack of the Fe-O oxygen centre by the C5 carbon of the thiazole ring. The presence of the amine group at the C2 position enhances the favourability of the epoxidation reaction; that is, epoxidation at aminothiazole (ATZ) is more favourable by 7.92 kcal/mol compared to epoxidation at the thiazole group.

It was found that four reactive metabolites (M1-M4) of thiazoles could rearrange to an additional ten isomers via simple chemical rearrangements, as shown in Figure 3. The global electrophilicity indices of these 14 reactive metabolites were also calculated. The calculations suggested that **I10** and **I11** are highly electrophilic, and hence, they cause toxicity.

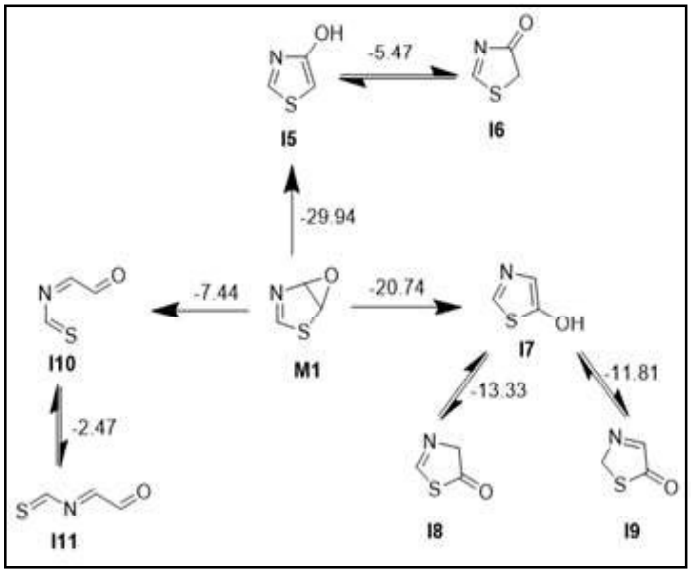


Figure 3. Isomeric forms of thiazole epoxide metabolite (M1) with rearrangeable processes and energy values in kcal/mol.

3. Artificial Intelligence in drug metabolism

Swamidas and coworkers[32] trained machine learning models using deep learning techniques to estimate quantum chemical properties. Transfer learning technology was used to conclude quantum chemical properties and to analyze chemical behaviour at the site level. This approach was employed in predicting the site of epoxidation and the site of covalent reactivity. The authors demonstrated that such a machine-learning approach could help explore the drug's metabolism.

Deep learning is one of the growing methods of artificial intelligence.[33] It can be applied to predict several aspects of the drug (i) toxicity, (ii) metabolism, (iii) bioactivity, (iv) mechanism of action, and thus valuable for drug design.[34] It has been established that very accurate quantum chemical properties can be estimated using deep learning methods. Quantum chemical studies are time-consuming because they involve the Schrodinger equation to solve the wavefunction to estimate the electronic structure of drugs and achieve a highly accurate level of prediction from quantum chemistry. Swamidas et al. adopted a two-stage machine learning approach. The deep learning method was used in the first stage, and in the second stage, the transfer learning method was used. In this approach, the deep neural network learns the observable properties of molecules similar to that of the wave function. Those calculated values can be transferred to another deep neural network to estimate drug metabolism and site-specific reactivity. The authors suggested that the deep learning approach using a small set of quantum chemical properties helps predict many more properties which are not employed in training.

The data set of epoxidation and reactivity were collected from previously published work by Hughes et al. Graph-based deep learning model was employed to compute quantum chemical representations. A message-passing neural network was constructed and trained using the PubChem QC project to predict the QC properties. Graph-based representations were computed for each atom in data sets collected for each model after training of PubChem QC data set. Two neural network models were developed used to perform transfer learning from quantum chemistry (TLQC) for both the reactivity data set and the epoxidation data set. The neural network was used to predict atomic level reactivity labels which had three layers incorporated into it. The first layer as input contained the information from the deep learning model, i.e., atom encodings, and this was passed to two hidden layers of 32 and 16 units and tanh activation. Finally, the information was passed through the output layer with four units and sigmoid activation. The next step performed by the authors was a comparison of models, topological descriptors (Top) or quantum chemical descriptors (QC). These models were used to predict both epoxidation and reactive sites. The 'neighbourhood convolution method' was used to construct the input details of the Top and QC models. The models were selected based on their highest overall accuracy across all tasks. The top-2 metric was chosen as the primary accuracy metric. Statistical tests like the McNemar test paired Z-test and Welch's t-test were also performed to validate the top-2 scores, AUCs and Z-scores.

By utilizing intermediate representations constructed through deep learning, transfer learning was

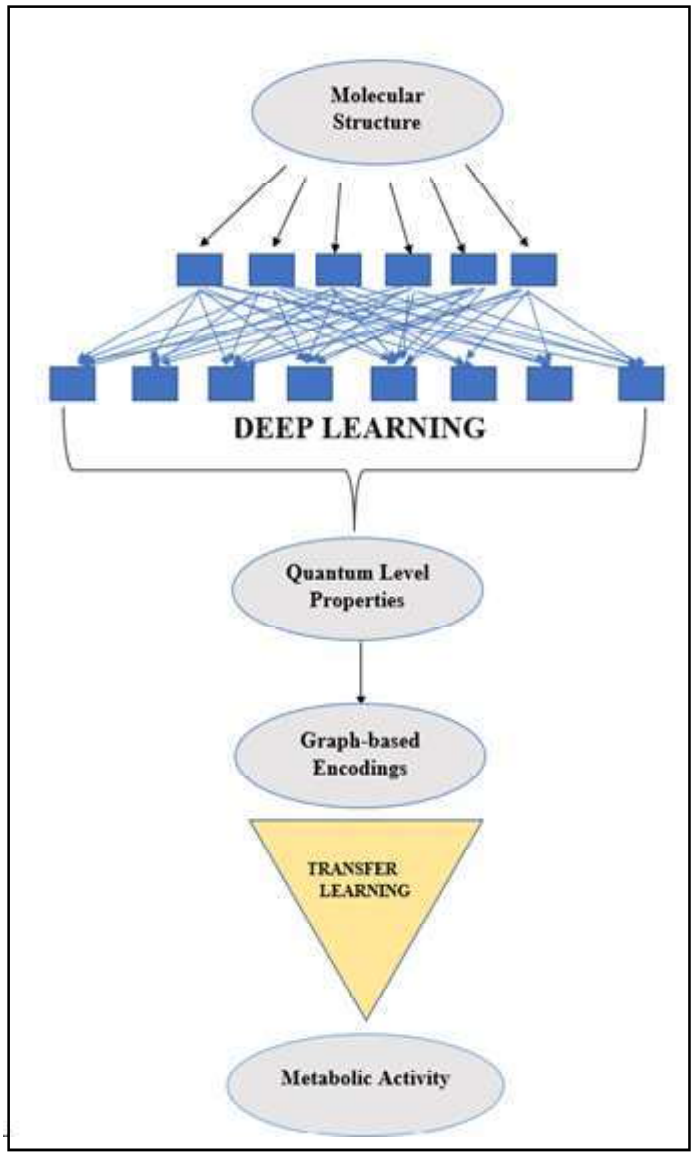


Figure 4. Flowchart depicting the steps involved in deep learning and transfer learning.

employed to accurately predict site-level chemical behaviours and compute additional quantum chemical properties. The prediction of the site of epoxidation was found to be more accurate for TLQC models than topological models. TLQC also showed better accuracy in three out of four reactivity tasks: cyanide, GSH, and protein covalent reactivity with the molecules. The results suggested that quantum representations may be generalizable to check the reactivity of other molecules apart from the properties of the QC set used to construct these representations. In comparison to topological models, TLQC yielded more accurate predictions for locations on an aromatic compound where epoxidation reactions can take place. Hence, this work suggested that transferring knowledge from the QC domain is fruitful in gaining reliable results and improving the models. In this way, a link was established by connecting the theory of quantum mechanics with chemical behaviours that are relevant to medicinal chemistry.

4. Chemoinformatics in drug metabolism

With the assistance of bacterial enzymes, drug metabolism has helped discover more than 50 drugs so far. Bacteria found in the human gut can activate, inactivate and reactivate drugs. This metabolism can show desirable or undesirable effects. It has been found that there are only a few computational tools for screening drugs that are metabolized by bacterial enzymes. Altman et al. designed a method to predict the metabolites of drugs using chemoinformatics descriptors and vector embedding techniques.[35] The significant obstruction for in-depth drug metabolism studies is analyzing time-consuming experiments like mass spectrometry. Mass spectrometry (MS) offers a quantitative analysis of metabolites with high sensitivity, selectivity, and the potential to identify metabolites. Traditional QSAR is also can be used to achieve this. It cannot depict the relationship between pairs of drugs and metabolites. It only focuses on an individual molecule. The metabolism of a drug molecule is defined over a pair of molecules. Hence, Altman et al. developed a novel computational approach for specifying the properties of molecular transformation. The central concept behind this approach is that there is a slight difference between metabolite and drug as the drug undergoes a well-defined transformation. For example, the hydroxylation of the drug upon getting catalyzed by CYP3A4, drug and metabolite forms matched molecular pairs. Many different approaches have been used for the matched molecular pair analysis (MMPA) method, like fragment indexing-based methods, structural and physiochemical approaches, etc. These approaches may fail to recognize multi-site transformations. The transformations are also considered independent of the surrounding atoms in these approaches.

Altman et al. tried to overcome these challenges by using the representations of chemical transformations as algebraic expressions of chemical structure vectors. The authors hypothesized that

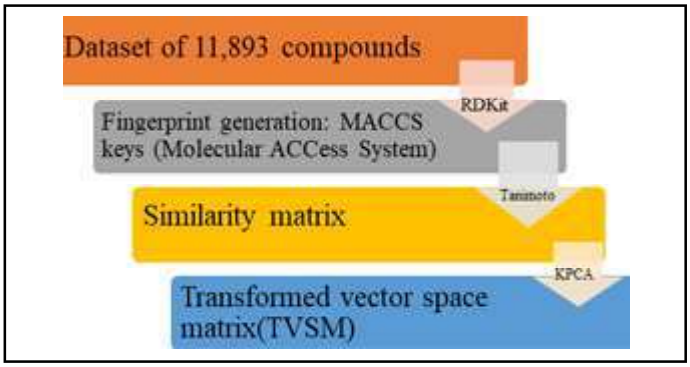


Figure 5. Schematic flow of vector space construction from a dataset

molecules in chemical reactions could form matching pairs with molecules of other reactions.

The data on compounds and chemical reactions were taken from the MetaCyc metabolic pathway database. The SMILES, reaction name, primary substrate compound, direction and immediate product were taken from the data collected. In total, 11,893 unique compounds were selected, which were included in a databank named compound_dataset with size num_compounds. The molecular vector space was generated after the selection of the compound dataset. The SMILES were taken as input that generated molecular fingerprints using kernel principal component analysis (KPCA). MACCS (Molecular ACCess System) key was used to encode molecular structure in a condensed bit vector form. Using molecular fingerprints, the Tanimoto similarity (Jaccard index) has been used as the kernel function for kernel PCA.

Further, the molecular-level and reaction-level analyses were done. K-means and enrichment analysis were used to find groups of given chemical types. Subsequently, the enriched molecule types for each cluster were determined using hypergeometric enrichment analysis with a Bonferroni correction. Kmeans clustering to reaction vectors formed using MetaCyc reactions was used to find the types of chemical reactions. Further enrichment was done to characterize clusters by enzymes that catalyze the reactions.

Queries were performed on metabolite-drug pairs inside a vector landscape. Similar reaction and drug-metabolite vectors were identified, which was a first step towards drug metabolism modelling using vector space. For example, one of the clusters contained a specific type of oxidoreductases and another group was enriched for glucosyltransferases. Hence, the authors discovered a method to compute similarities between the biotransformation of drugs and chemical reactions.

5. Conclusions

This assessment is centred on establishing the value of pharmacoinformatics efforts to understand the molecular mechanism of drug metabolism. Predicting the drug metabolism using AI and chemoinformatics tools prior to the in vitro experiments and the detailed molecular mechanism using quantum chemical studies can save time and resources. In this review, three case studies have been discussed, which show the importance of i) Quantum chemical studies, ii) Artificial intelligence iii) Chemoinformatics tools.

6. Acknowledgements:

Prof. P.V. Bharatam is very thankful to DBT, India for providing financial support (BT/PR40164/BTIS/137/17/2021) in NIPER, S.A.S. Nagar, India.

References

1. De Groot, M. J. (2006) Designing better drugs: predicting cytochrome P450 metabolism. Drug Discov. Today, 11, 13-14
2. Jancova, P.; Anzenbacher, P.; Anzenbacherova, E. (2010) Phase II drug metabolizing enzymes. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub, 154, 103-116.
3. Gibson, G. G.; Skett, P. (2013) Introduction to drug metabolism; Springer.
4. Dixon, R. L.; Shultice, R. W.; Fouts, J. R. (1960) Factors affecting drug metabolism by liver microsomes. IV. Starvation. Proc. Soc. Exp. Biol. Med. 103, 333-335
5. Vesell, E. S.(1968) Genetic and environmental factors affecting hexobarbital metabolism in mice. Ann N Y Acad Sci, 151, 900-912.
6. Taxak, N.; Bharatam, P. V. (2014) Drug metabolism. Resonance 19, 259-282.
7. Grillo, M. P. (2015) Detecting reactive drug metabolites for reducing the potential for drug toxicity. Expert Opin. Drug Metab. Toxicol, 11, 1281-1302.
8. Ramesh, M.; Bharatam, P. V. (2014)Importance of hydrophobic parameters in identifying appropriate pose of CYP substrates in cytochromes. Eur. J. Med. Chem., 71, 15-23.
9. Kohn, W.; Becke, A. D.; Parr, R. G.(1996) Density Functional Theory of Electronic Structure. The Journal of Physical Chemistry, 100 (31), 12974-12980.
10. Jaladanki, C. K.; Shaikh, A.; Bharatam, P. V. (2017) Biotransformation of Isoniazid by Cytochromes P450: Analyzing the Molecular Mechanism using Density Functional Theory. Chem. Res. Toxicol., 30 (11), 2060-2073.
11. Warren, T. K.; Jordan, R.; Lo, M. K.; Ray, A. S.; Mackman, R. L.; Soloveva, V.; Siegel, D.; Perron, M.; Bannister, R.; Hui, H. C.; et al. (2016) Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature, 531 (7594), 381-385.
12. Beigel, J. H.; Tomashek, K. M.; Dodd, L. E.; Mehta, A. K.; Zingman, B. S.; Kalil, A. C.; Hohmann, E.; Chu, H. Y.; Luetkemeyer, A.; Kline, S.; et al.(2020) Remdesivir for the Treatment of Covid-19 - Final Report. N. Engl J. Med., 383 (19), 1813-1826.
13. Williamson, B. N.; Feldmann, F.; Schwarz, B.; Meade-White, K.; Porter, D. P.; Schulz, J.; van Doremalen, N.; Leighton, I.; Yinda, C. K.; Perez-Perez, L.; et al. (2020) Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature, 585 (7824), 273-276.
14. Siegel, D.; Hui, H. C.; Doerffler, E.; Clarke, M. O.; Chun, K.; Zhang, L.; Neville, S.; Carra, E.; Lew, W.; Ross, B.; et al. (2017) Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo[2,1-f][triazin-4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses. J. Med. Chem., 60 (5), 1648-1661.
15. Gordon, C. J.; Tchesnokov, E. P.; Woolner, E.; Perry, J. K.; Feng, J. Y.; Porter, D. P.; Gotte, M. (2020)Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. J. Biol. Chem., 295 (20), 6785-6797.

16. Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. (1999) Characterization of the activation pathway of phosphoramidate triester prodrugs of stavudine and zidovudine. Mol. Pharmacol., 56 (4), 693-704.
17. Zhang, L.; Zhou, R. (2020) Structural Basis of the Potential Binding Mechanism of Remdesivir to SARS-CoV-2 RNA-Dependent RNA Polymerase. J. Phys. Chem. B 124 (32), 6955-6962.
18. Jung, L. S.; Gund, T. M.; Narayan, M. (2020) Comparison of Binding Site of Remdesivir and Its Metabolites with NSP12-NSP7-NSP8, and NSP3 of SARS CoV-2 Virus and Alternative Potential Drugs for COVID-19 Treatment. Protein J 39 (6), 619-630.
19. Wang, Y.; Zhang, D.; Du, G.; Du, R.; Zhao, J.; Jin, Y.; Fu, S.; Gao, L.; Cheng, Z.; Lu, Q.; et al. (2020) Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet, 395 (10236), 1569-1578.
20. Kapoor, S.; Dubey, G.; Khatun, S.; Bharatam, P. V. (2022) Remdesivir: Mechanism of Metabolic Conversion from Prodrug to Drug. Curr. Drug Metab., 23 (1), 73-81
21. Kalgutkar, A. S.; Didiuk, M. T. (2009) Structural alerts, reactive metabolites, and protein covalent binding: how reliable are these attributes as predictors of drug toxicity? Chem. Biodivers., 6 (11), 2115-2137.. Kalgutkar, A. S.; Fate, G.; Didiuk, M. T.; Bauman, J. Toxicophores, (2008) reactive metabolites and drug safety: when is it a cause for concern? Expert Rev Clin Pharmacol, 1 (4), 515-531.
22. Stachulski, A. V.; Baillie, T. A.; Park, B. K.; Obach, R. S.; Dalvie, D. K.; Williams, D. P.; Srivastava, A.; Regan, S. L.; Antoine, D. J.; Goldring, C. E.; et al. (2013) The generation, detection, and effects of reactive drug metabolites. Med. Res. Rev 33 (5), 985-1080.
23. Kalgutkar, A. S.; Obach, R. S.; Maurer, T. S. (2007) Mechanism-based inactivation of cytochrome P450 enzymes: chemical mechanisms, structure-activity relationships and relationship to clinical drug-drug interactions and idiosyncratic adverse drug reactions. Curr. Drug. Metab., 8 (5), 407-447.
24. Walsh, J. S.; Miwa, G. T. (2011) Bioactivation of drugs: risk and drug design. Annu. Rev. Pharmacol. Toxicol. 51, 145-167.
25. Obach, R. S.; Kalgutkar, A. S.; Ryder, T. F.; Walker, G. S. (2011) In vitro metabolism and covalent binding of enol-carboxamide derivatives and anti-inflammatory agents sudoxicam and meloxicam: insights into the hepatotoxicity of sudoxicam. Chem. Res. Toxicol., 21 (9), 1890-1899.
26. Mizutani, T.; Yoshida, K.; Kawazoe, S. (1994) Formation of toxic metabolites from thiabendazole and other thiazoles in mice. Identification of thioamides as ring cleavage products. Drug Metab. Dispos, 22 (5), 750-755.
27. Mizutani, T.; Suzuki, K.(1996) Relative hepatotoxicity of 2-(substituted phenyl)thiazoles and substituted thiobenzamides in mice: evidence for the involvement of thiobenzamides as ring cleavage metabolites in the hepatotoxicity of 2-phenylthiazoles. Toxicol. Lett. 85 (2), 101-105.
28. Kalgutkar, A. S.; Driscoll, J.; Zhao, S. X.; Walker, G. S.; Shepard, R. M.; Soglia, J. R.; Atherton, J.; Yu, L.; Mutlib, A. E.; Munchhof, M. J.; et al. (2007) A rational chemical intervention strategy to circumvent bioactivation liabilities associated with a nonpeptidyl thrombopoietin receptor agonist containing a 2-amino-4-arylthiazole motif. Chem. Res. Toxicol, 20 (12), 1954-1965.
29. Subramanian, R.; Lee, M. R.; Allen, J. G.; Bourbeau, M. P.; Fotsch, C.; Hong, F. T.; Tadesse, S.; Yao, G.; Yuan, C. C.; Surapaneni, S.; et al. (2010) Cytochrome P450-mediated epoxidation of 2-aminothiazole-based AKT inhibitors: identification of novel GSH adducts and reduction of metabolic activation through structural changes guided by in silico and in vitro screening. Chem. Res. Toxicol., 23 (3), 653-663.
30. Jaladanki, C. K.; Khatun, S.; Gohlke, H.; Bharatam, P. V.(2021) Reactive metabolites from thiazole-containing drugs: quantum chemical insights into biotransformation and toxicity. Chem. Res. Toxicol. 34 (6), 1503-1517.
31. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; et al.(2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J. Med. Chem., 47 (7), 1739-1749.
32. Sarullo, K.; Matlock, M. K.; Swamidass, S. J. A.(2020) Site-level bioactivity of small-molecules from deep-learned representations of quantum chemistry. J. Phys. Chem. A 124 (44), 9194-9202.
33. Kelleher, J. D. Deep learning; MIT press, 2019.
34. Hughes, T. B.; Swamidass, S. J. (2017) Deep Learning to Predict the Formation of Quinone Species in Drug Metabolism. Chem. Res. Toxicol., 30 (2), 642-656.
35. Mallory, E. K.; Acharya, A.; Rensi, S. E.; Turnbaugh, P. J.; Bright, R. A.; Altman, R. B.(2018) Chemical reaction vector embeddings: towards predicting drug metabolism in the human gut microbiome. In PACIFIC SYMPOSIUM ON BIOCOMPUTING Proceedings of the Pacific Symposium.

CRIPS Digest

Semaglutide: GLP-1 agonist in the treatment of Non-alcoholic Steatohepatitis

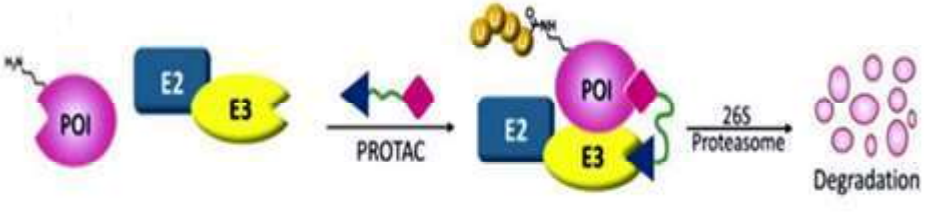
Semaglutide, a potent GLP-1 agonist which has been approved for the treatment of type 2 diabetes mellitus, recently showed protective effect against Non-alcoholic Steatohepatitis (NASH). It is a longer acting GLP-1 receptor agonist with 94% structural similarity with native USFDA approved GLP-1 agonists which are available as Ozempic (s.c injection, weekly once dosing with 0.5 and 1 mg strength) and Rybelsus (oral tablet, once daily dosing in 3, 7 and 14 mg strength). NASH is associated with disruption in insulin signaling, lipid peroxidation, activation of inflammatory pathways and liver injuries and Semaglutide shows indirect protective effect through gut-pancreases-liver axis. This drug showed protective role by modulating hepatic mitochondrial function, increasing insulin sensitivity and reducing accumulation of free fatty acid. In a phase 2 clinical trial, Semaglutide (s.c, daily dose of 0.1, 0.2 and 0.4 mg strength) improved the NASH by 40%, 30% and 15% respectively as compared to the placebo and even without worsening the fibrosis. In other placebo-controlled phase 2 study, similar Semaglutide (given in s.c weekly once dose) in 65 subjects and on MRI study it shows protective effect by ameliorating the liver fibrosis, change in liver stiffness and liver fat content. In a phase-3 clinical trial Semaglutide improved cirrhosis, resolution of steatohepatitis and histology of balloon shaped hepatocyte in NAH patients, however without cirrhosis. So Semaglutide can be given in patient as monotherapy or along with dietary restriction and exercise for the treatment of NASH. (Nat. Rev. Immun., (2022) 22: 429-443)

Discovery of Pyrrolopyrimidine Derivatives as Selective Perinucleolar Compartment (PNC) inhibitors, a Phenotypic Marker of Tumor Progression

Cancer, one of the major health problems and second leading cause of death globally is still a serious threat to human health worldwide, despite the development of various treatments. It involves a process by which primary tumors disseminate throughout the body to secondary sites, which happens to be the foremost cause of mortality for >90% of cancer patients. To overcome this, a phenotypic marker perinucleolar compartment (PNC), which can identify cancer cells competent to metastasize and correlate with cancer progression and metastatic capacity, make it a useful marker for metastatic cancer progression. The PNC is a membrane-less, highly dynamic subnuclear body found at the periphery of the nucleolus. It is enriched with RNA transcripts and RNA-binding proteins, reflecting different states of genome organization. It forms only in cancer cells but not in normal, non-transformed cells including embryonic stem cells. The authors carried out the work identifying and developing novel compounds that reduce PNC prevalence at concentrations where cell viability is not affected. They performed detailed medicinal chemistry optimization campaign around a pyrrolopyrimidine series that ultimately led to the discovery of the bioavailable analogue, metarrestin (NCATS-SM0590), which has shown potent antimetastatic activity with improved survival in rodent models and is currently being evaluated in a first-in-human phase 1 clinical trial. (J. Med. Chem. 2022, 65, 8303; Sci. Transl. Med. 2018, 10, 8307.).

PROTAC as heterobifunctional modality in drug discovery

PROTACs or proteolysis targeting chimeras is a strategy that utilizes ubiquitin proteosome system to target a specific protein which lead to its degradation in the cell. PROTACs are hetero bifunctional molecules that connect a protein of interest ligand to an E3 ubiquitin ligase (E3) recruiting ligand with an optimal linker. Nowadays PROTAC technology is used to target varieties of proteins including transcription factors, skeleton proteins, enzymes, and regulatory proteins. This technology is used to treat various diseases like cancer, viral infections, immune disorders and neurodegenerative disorders. This is mainly due to the potent ability of PROTACS to induce targeted protein degradation



by using body's ubiquitin proteasome system. It is not possible to develop drugs or viable small molecules that act on undruggable targets like transcription factors, IKZF1/3, CSNK1A1, ZFP91 that inhibit interactions of endogenous proteins or nucleic acids on these targets. By using PROTACs we are artificially mimicking this process to carry out degradation of desired protein.

Steps involved in PROTAC technology

1. The PROTAC binds both the target protein and the E3 ligase simultaneously to induce the formation of a ternary complex.
2. The target protein is then polyubiquitinated by E3 ligase.
3. Proteolysis of protein of interest by 26S Proteasome.

There are different PROTACs based on the different types of E3 ubiquitine ligases like ring family E3s, Culling ring E3s, Von Hippel-Lindau, cereblon (CRBN), Cellular Inhibitor of apoptosis (cIAP) and Mouse Double Minute 2 homolog (MDM2).

ARV 110 and ARV 471 are in clinical trial phase ?? for the treatment of prostrate cancer and breast cancer respectively. (Nat. Rev. Drug Discovery. 2022, 21, 3, 181-200; J. Med. Chem. 2018, 61, 2, 444-452.)

Programmed genome editing by a miniature CRISPR-Cas12f nuclease.

The CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat- CRISPR-associated protein) system has been employed as a system of adaptive immunity in bacteria and archae. It found increasing use as a gene editing tool in laboratory as well as clinical setting. However, their large size makes their encapsulation in vectors with limited size difficult. Wu et al. report the characterization of Acidibacillus sulfuroxidans Cas12f1 (AsCas12f1) (422 amino acids), an example of a miniature type V-F effector protein. Type V-F class (Cas12f) is characterized by small Cas effector proteins (400-700 residues). The protospacer adjacent motifs (PAM) specificity of CRISPR-AsCas12f1 was determined by subtractive high throughput screening of an E. coli library of randomized PAM sequences. PAM depletion assay showed that AsCas12f1 effector recognized PAMs as 5?-NTTR (R: A/G). The RNA component of AsCas12f1 was identified by small RNA seq to be mature crRNA (45-48 nt) and probable tracrRNA (138-144 nt). Fluorescence labelling revealed the putative dsDNA substrate which was degraded with the generation of 11 nt 5? overhang at target strand near the PAM. A non-cohesive end was generated at the segment away from the protospacer. AsCas12f1 also recognized ssDNA and cleaved it at the same site as dsDNA but this effect was not dependent on PAM. The ability of the screened Cas effector to carry out gene manipulation in bacteria was monitored in K. pneumoniae. Both drug-resistant and metabolic genes could be disrupted. AsCas12f1 was also able to accomplish gene editing in HEK293 cells where an inactivated EGFP expression cassette could be activated by restoration of the reading frame. AsCas12f1 is the smallest programmable endonuclease currently in use for human cell editing and allows its packaging into adeno-associated viral vector. The encapsulated CRISPR-AsCas12f1 system was able to edit targeted genes in different cell lines. (Wu Z, Zhang Y, Yu H, Pan D, Wang Y, Wang Y, Li F, Liu C, Nan H, Chen W, Ji Q. Programmed genome editing by a miniature CRISPR-Cas12f nuclease. (Nat. Chem. Biol. 2021, 17, 1132-1138).