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# FROM THE EDITOR

“

It is my privilege to publish the 39th edition of PHARMBIT, the esteemed journal of the Department of Pharmaceutical Sciences and Technology, on the behalf of Pharmaceutical Society. As an annual scholarly publication, our aim is to disseminate novel research related to pharmacy and life sciences. PHARMBIT is currently abstracted in several renowned databases, including Natural Science Database, ProQuest, Index Copernicus, Google Scholar, ASCI-Database and CiteFactor.

In this 39$^{th}$ volume, we present five manuscripts by authors from around the world covering a range of timely topics. These include the versatile pharmacological properties of oxazole, *in silico* techniques for drug discovery including artificial intelligence and machine learning, comorbidities associated with COVID-19, and colistin resistance mechanisms in microorganisms. The global crisis of antibiotic resistance threatening millions of lives annually remains a pivotal area of research for the scientific community. As members of the pharmaceutical research community, it is our duty to provide solutions to society's most pressing medical needs. The present edition of PHARMBIT attempts to address these critical problems and propose innovative solutions.

As Chief Editor, I want to sincerely thank all the authors who have chosen to publish their manuscripts with us. My gratitude also goes out to our esteemed Editorial Board for their guidance and expertise, as well as the HOD and faculty of our department for their enduring support. The leadership of our Vice Chancellor has been instrumental in sustaining this invaluable publication legacy of the Department of Pharmaceutical Sciences and Technology

I hope these rigorously peer-reviewed contributions will further knowledge in their respective domains and provide inspiration for future pharmaceutical research worldwide. Please enjoy this edition of PHARMBIT.

”

Sincerely,

*Manik Ghosh*

Chief Editor

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# Impact of Hypertension Comorbidity and Renin Angiotensin Aldosterone System Inhibitor Drugs on COVID-19 Patients: An Overview

**Pargat Singh, Gagan Deep Longowal, Gaurav Chaudhary, Amritpal Kaur, Diksha Choudhary and Rajwinder Kaur***

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**A B S T R A C T**

In late 2019, Chinese healthcare professionals reported cases of unknown disease with pneumonia symptoms. A virus that rapidly spread worldwide was identified and named SARS-CoV-2 (COVID-19). The comorbidities (especially hypertension) were major topics of discussion after analyzing them in most COVID-19 individuals. Hypertension directly affects the severity alone or along with the Renin Angiotensin Aldosterone System (RAAS) inhibitors used to treat hypertension. RAAS Inhibitors has both positive and negative impact on COVID-19 patients' susceptibility and outcomes. Hypertension comorbidity affects the outcome of COVID-19 patients. In this article, we tried to discuss the link between COVID-19 and hypertension and RAAS inhibitors. To find the exact relation of COVID-19 with RAAS inhibitors, one must collect large data, statistically analyze, and properly compile the result, using appropriate tools.

**Keywords:** ACEIs/ARBs, COVID-19, Diseases, SARS-CoV-2, Health, Hypertension, Mortality, RAAS inhibitors.

## 1. INTRODUCTION

Coronavirus is a single-stranded RNA virus belonging to the Coronaviridae family [1, 2], whose earlier cases were seen at the ending days of the year 2019 with some symptoms of lower respiratory tract infection [1, 3]. This rapid-spreading virus, which has spread almost all around the world with a total of more than 1,84,56,665 cases [4] was identified and named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) commonly called COVID-19 [3, 5]. In 2012, more than 2,200 similar cases were seen all around the globe that caused mortality in more than 33% of individuals [6]. These viruses are thought to be spread from many avian and mammals [2].

Acute Respiratory Distress Syndrome (ARDS) is seen in severe cases of SARS-CoV-2, requiring intensive care and mechanical ventilation [7]. This SARS-CoV-2 is thought to be very virulent for the geriatrics, the immune-compromised [8], and for the individuals who are already suffering from some chronic illnesses like Cardiac disease, Diabetes, etc., of the which most common is Hypertension (HTN), followed by Diabetes and Cardiovascular Diseases [9-12]. HTN is a chronic illness. When a person's blood pressure is more than 140/90 mm of Hg, he/she is said to be hypertensive [13]. More than 1.39 billion individuals around the globe are suffering from HTN [14, 15]. HTN is so prevalent in low-and-middle-income nations that they account for 75% of whole hypertensive cases [15, 16]. According to the World Health Organization,

less than 1 out of 5 are having good control over it [17].

## 2. COMMON COMORBIDITIES IN COVID-19 PATIENTS

Being old and having comorbidities are prominent features of getting infected by SARS-CoV-2. The US adult population statistics show that 60% of hospitalized COVID-19 patients had a condition [18]. HTN, Diabetes, and Cardiovascular diseases are the most usual comorbidities among COVID-19 patients. A study shows that Diabetes along with HTN, treated using RAAS inhibitors, is frequent comorbidity with more mortality probability and may often need intensive care unit (ICU) administration [19, 20]. Out of 72,314 cases reported, mortality in hypertensive patients increases from 2.3% to 5% and in diabetic patients, it increases to 7.3% [21]. These kinds of patients need close monitoring [22]. Li, X. *et al.* regarding these comorbidities, studied 25 deceased individuals (10 males, 15 females), who were treated for 4-20 days, and data are given in **Table 1** along with other studies [23]

**Table 1:** Most common comorbidities in COVID-19 Patients

| TOTAL PTS* | % HTN* | % DIABETES | % CVD* | REFERENCE |
|---|---|---|---|---|
| 419 | 40.5 | 15.2 | 6.2 | [24] |
| 2,209 | 20.7 | 10.5 | 7.4 | [22] |
| 355 | — | 35.5 | 42.5 | [22] |
| 25 | 64.0 | 40.0 | 30.0 | [23] |
| 310 | 36.5 | 15.5 | 6.1 | [3] |
| 1590 | 16.9 | 8.2 | 3.7 | [12] |
| — | 57.1 | 38.8 | 41.0 | [21] |

HTN-Hypertension, CVD-Cardiovascular Disease, PTS-Patients

A study on 1,590 patients in Wuhan, China reflects that 20-51% of patients have at least one comorbidity and these comorbidities elevate ARDS risk by 3.4 times [12]. Alanazi *et al.* studied 32 patients and concluded that all patients, who have diabetes without HTN and who have neither diabetes nor HTN, survived [6].

## 3. COMORBIDITIES IN COVID-19 PATIENTS

### *3.1 Cardiovascular comorbidities:*

Cardiovascular diseases are the most frequent and severe in COVID-19 individuals [24]. These patients are suffering from COVID-19 complications because the virus is capable of disrupting the stabilized atherosclerotic plaque and cardiovascular system [8, 11, 20].

CVD causes Renin-angiotensin system imbalance, inflammatory storm, hypoxemia, and stress responses that are why, early symptoms in these COVID-19 patients were palpitations, chest tightness, followed by heart failure or myocardial infarction [11, 20]. In young hypertensive COVID-19 positive patients recovered after anti-viral therapy ultimately severe respiratory distress syndrome and cardiac insufficiency development are seen [25].

A review by Bajgain *et al.* reflects that acute cardiac injury is seen in 7-28% of all COVID-19 individuals and these injuries result in mortality in almost 10.5 % (versus 0.9% without comorbidity) of individuals [26]. Roncon *et al.'s* investigation in Italy reflects that arterial HTN is commonly seen in expired COVID-19 ICU patients [27].

### *3.2. Other comorbidities:*

Pulmonary arterial hypertension (PAH) is very uncommon in COVID-19 patients. 10 cases of PAH were reported by Nuche *et al.* [28]. Seven out of ten (7/10) individuals were hospitalized for an average of 10 days, 5 out of them required Oxygen therapy but none of them need intensive care administration [28]. Along with common comorbidities, Guan *et al.* explained other comorbidities as well [12]. The percentage of other comorbidities in COVID-19 patients were Cerebrovascular disease (1.9%), COPD (1.5%), CKD (1.3%), Malignancy (1.1%), and Immunodeficiency (0.2%) [12].

## 4. GENERAL ANTI-HYPERTENSIVE AGENTS

Diuretics (Thiazide, Potassium-Sparing, Loop), Angiotensin-converting enzyme inhibitors (ACEIs), Angiotensin Receptor Blockers (ARBs), Beta-Blockers (Beta-1 Selective, Alpha activity), Vasodilators, Calcium Channel Blockers, Selective Aldosterone Antagonists, Alpha2-agonists (Central-acting), Renin Inhibitors (Combos), Antihypertensive Alpha Blockers, Other Antihypertensives (like Reserpine) are the different classes of drugs that are used in the treatment of HTN [29]. An analysis of most common drugs used in Hypertension and heart failure treatment reflects frequent usage of ACEIs and ARBs [30].

## 5. IMPACT OF HYPERTENSION COMORBIDITY ON COVID-19 PATIENTS

HTN was frequently seen in the early cases of COVID-19 [31], due to which it seeks the attention of healthcare professionals. HTN, over time, ruptures the blood vessels and reduces the blood volume reaching the heart. It leads to unfavorable results in COVID-19 individuals [32]. HTN frequency in 5,700 US hospitalized COVID-19 patients was 56%, similar to China (50%) and Italy (49%) (**Table 2**).

**Table 2:** Percentage of COVID-19 patients having HTN comorbidity

| S. No. | Country | % Hypertensive patients | Reference |
|---|---|---|---|
| 1 | — | 16.3 & 17.1 | [1] |
| 2 | Italy | 49.0 | [5] |
| 3 | US | 56.0 | [5] |
| 4 | China | 50.0 | [5] |
| 5 | China | 17.0 | [10] |

According to Liang *et al.*, HTN had a remarkable link with mortality and adverse outcomes in COVID-19 individuals [33], and Gao *et al* .supported this study by stating that HTN increases the risk of mortality by 2 folds [34]. Arterial HTN has a bad effect on COVID-19 infection [35]. The exact relation between HTN and COVID-19 is not clear [22, 32], but the laboratory investigations in hypertensive individuals (comparing to normotensives) reflects high levels of Leukocytes, Neutrophils, Neutrophil/lymphocytes ratio [3, 31, 33, 36], liver function tests (Total Bilirubin, SGOT, SGPT), and Renal function tests (serum creatinine and BUN), Creatine kinase, LDH, and C-reactive proteins; low levels of

lymphocytes, liver albumin, serum potassium and serum sodium [37]. High levels of D-dimer [36] and other causes could be impaired immune system (low levels of CD3, CD4, CD8 T-cells, imbalance of cytokines (cytokines storm) [3, 10, 36]. Hu *et al.* observed that hypertensive patients require more antibiotics, hormonal, and *i.v.* immunoglobulin treatments, ICU admission, and hospital stay (**Table 3**) [31].

**Table 3:** Effect of HTN on various aspects of COVID-19

| Impact on severity? | Increase ICU admission? | Adverse outcomes? | High Mortality risk? | Reference |
|---|---|---|---|---|
| Yes | Yes (2 folds) | - | Yes | [1] |
| - | Yes | - | Yes | [38] |
| - | Yes | - | - | [24] |
| - | - | - | Yes | [27] |
| Yes | - | Yes | Yes | [31] |
| - | - | Yes | Yes | [33] |
| unclear | - | - | Yes | [22] |
| Yes | Yes | - | Yes | [3] |
| - | - | - | Yes | [34] |

## 6. IMPACT OF RENIN ANGIOTENSIN ALDOSTERONE SYSTEM INHIBITORS (RAASIs) ON COVID-19

Renin-Angiotensin-Aldosterone System (RAAS) plays an important role in maintaining blood pressure, hydro-electrolyte balance, and pathogenesis of inflammatory diseases (Figure 1) [39]. Some studies state that HTN may not be the cause, but drugs used to treat HTN might be playing a crucial role in COVID-19 patients, especially ACEIs, ARBs, and RAASIs as they increase the number of ACE-2 receptors who are binding sites for COVID-19 [32, 40] virus to enter a host cell, in contrast, ACE-2 (with the help of RAAS-Is) plays protective roles in Pneumonitis (lung inflammation) and lung injury [41-44]. In concern, ACEIs and ARBs can reflect both positive and negative effects by increasing susceptibility and by improving lung injury in COVID-19 [45].

### *6.1. Susceptibility to COVID-19:*

The susceptibility of COVID-19 disease in ACEIs and ARBs is similar to other Anti-hypertensive drugs [46, 47] and no direct evidence reflects COVID-19 likelihood in individuals taking RAASIs was found [11, 15, 48].

### *6.2. Impact on Severity and Outcome:*

Pinto-Sietsma *et al.* reflect no severity difference in COVID-19 individuals [32] and RAASIs had no impact on the severity and clinical outcomes [10, 31, 38].

### *4.5. Impact on mortality:*

Different studies are showing different outcomes regarding mortality. Edmonston *et al.'s* studies reflect both increase and decrease in mortality after use of RAASIs [42] whereas studies by Drager *et al.*, Pranata *et al.*, and Gupta *et al.* show no relation [48-50]. Williams, B. *et al.*, and Zaki, N. *et al.* state that the mortality rate was low in RAASIs as compared to other antihypertensive agents [19, 38]. Gao, C. *et al.* state that there is no difference in the proportion of ventilated patients between

RAASIs patients and patients using other anti-hypertensive agents but RAASIs tend to increase the risk of mortality [34].

According to Cui *et al.*, ACEIs/ARBs do not result in any adverse effect, did not affect symptoms, lab investigations, and prognosis in COVID-19 individuals [51]. American Heart Association announces "not to add or remove any RAAS related treatments, beyond action based on standard clinical practice" [37]. Bosso, M. *et al.* supported this statement by showing no harmful impact of RAAS Inhibitors [43]. The ACC, Heart Failure Society of America suggested to treat the patients with ongoing anti-hypertensive therapy [5, 11, 37, 45] as withdrawal of RAASIs can cause a stroke or heart attack, and hospitalization would increase COVID-19 exposure [8]

Some studies reflect the blocking action of Calcium-channel blockers on viral replication [38, 47], so these drugs can also be used. Also, the beneficial role of Beta-blockers was seen in COVID-19 patients [32]. In Italy, out of overall mortality, 69.1% of patients were hypertensive (30% administering ACEIs and 17% administering ARBs). But another data shows that the mortality rate in ACEIs/ARBs administering patients was less (3.7%) as compared to other anti-hypertensives (9.8%) [10].

## 7. CONCLUSION

Earlier observations of HTN comorbidity in COVID-19 cases along with the association between RAASIs and ACE-2 receptor (main pathway for the SARS-CoV-2 entry into the host cell) charge up the research work on the hypertensive patients. HTN is one of the world's leading chronic diseases and its impact increases with age. In our study, almost 75% of papers are indicating the positive association between HTN and COVID-19 severity (15 out of 20 papers). Again, we compiled the data to know the impact of RAASIs administration on COVID-19 susceptibility and outcomes. RAASIs had both good and bad impacts on COVID-19 individuals. Almost every study state that the impact of RAASIs is just a hypothesis with no or even slight positive effect on COVID-19 outcomes. Only a few studies show a negative impact on outcomes. To find the exact relation of COVID-19 with RAAS-Is, one must collect large data, statistically analyze, and correctly compile the result, using appropriate tools.

## 8. CONFLICT OF INTEREST

The author declares no conflict of interest.

## 9. ACKNOWLEDGEMENTS

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## 10. AUTHOR'S CONTRIBUTIONS

Rajwinder Kaur has suggested the concept and design for the review article preparation. Pargat Singh, Gagan Deep Longowal and Gaurav Chaudhary searched and collected the data. Pargat Singh performed the review of the collected articles, combined and drafted the manuscript with the help of Gagan Deep Longowal. Amritpal Kaur contributed to the figure and tables. Sandeep Arora with all authors helped in critical revision of the manuscript for imperative intellectual content. All authors read and approved the final manuscript

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# Oxazole Derivatives as Versatile Platform in Medicinal Chemistry: Synthesis, Biological Activities, and Therapeutic Potential

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**A B S T R A C T**

In the fields of organic and medicinal chemistry, oxazole a heterocyclic molecule with a five-membered ring that has one oxygen and one nitrogen atom has attracted a lot of interest. This mini-review offers a succinct synopsis of the structural characteristics, biological activity, and synthesis of oxazole derivatives. Several techniques, including cyclization reactions, condensation processes, and rearrangements, are used in the synthesis of oxazole to provide a flexible scaffold. The distinct structural features of oxazole enable a wide range of uses in the design and development of bioactive compounds. A wide range of pharmacological actions, such as antibacterial, antiviral, anticancer, and anti-inflammatory effects, have been shown for oxazole derivatives. The goal of this mini-review is to present a thorough summary of the current state of knowledge about oxazole and its derivatives, including information on their structural properties, biological activity, and synthesis.

**Keywords:** Antibacterial, Antiviral, Anticancer, Heterocyclic Compound, Oxazole.

## 1. INTRODUCTION

Oxazole **(Figure 1)** is a heterocyclic molecule with one oxygen and one nitrogen atom that is a member of the five-membered aromatic ring family. Oxazole's molecular structure is made up of a four-carbon ring with nitrogen and carbon atoms alternating, as well as an oxygen atom next to one of the nitrogen atoms. Because of its special configuration, oxazole has unique chemical and physical characteristics, which makes it an important motif in the fields of organic and medicinal chemistry.**[1]**

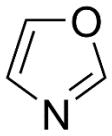


**Figure 1**: Oxazole structure containing one nitrogen, one oxygen, and three carbon atoms.

## 2. SYNTHESIS

### *2.1. Robinson-Gabriel Synthesis:*

The Robinson-Gabriel synthesis is a widely utilized technique for the synthesis of oxazoles. It involves subjecting an a-acylamino ketone to cyclization and dehydration through treatment with PCl5 or a strong mineral acid. Particularly useful for the synthesis of 2,5-diaryloxazoles **(Figure 2)**, which are now of interest in our research on the interactions of

heterocyclic systems with singlet oxygen, is this synthesis.**[2]**

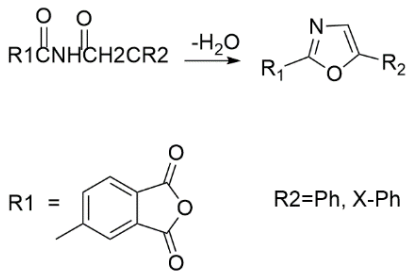


**Figure 2**: The Robinson-Gabriel Synthesis. An a-acylamino ketone to cyclization and dehydration through treatment with $PCl_5$ or any strong mineral acid.

### *2.2. Fischer Oxazole Synthesis:*

The Fischer oxazole synthesis involves the chemical reaction of cyanohydrin and aldehyde with anhydrous hydrochloric acid to produce an oxazole. Emil Fischer made this discovery in 1896. The actual cyanohydrin is made from a different aldehyde. The cyanohydrin of one aldehyde and the other aldehyde itself are the reactants of the actual oxazole synthesis and are often present in equimolar levels **(Figure 3)**. An aromatic group typically exists in both reactants and appears at particular locations on the resultant heterocycle.**[3]**

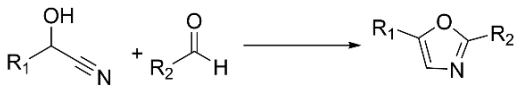


**Figure 3:** The Fischer Oxazole Synthesis. The cyanohydrin of one aldehyde and the other aldehyde itself are the reactants of the actual oxazole synthesis and are often present in equimolar levels.

## 3. REACTION

Oxazole is a five-membered aromatic heterocycle with one nitrogen and one oxygen atom. Its reactivity varies depending on whether or not it is functionalized and whether or not it has substituents. The following are a few typical reactions linked to oxazole:

### *3.1 Electrophilic Aromatic Substitution (EAS):*

Since it is an aromatic molecule, electrophilic aromatic substitution reactions can occur with oxazole. This entails adding an electrophile to the oxazole ring in place of a hydrogen atom. Certain places in the ring are more reactive than others due to the presence of nitrogen and oxygen atoms.**[4]**

### *3.2. Nucleophilic Substitution:*

Depending on the electrophilic character of the substrate, oxazole can go through several positions during nucleophilic substitution reactions. Under some circumstances, the nitrogen atom can function as a nucleophile.

### *3.3. Ring Opening Reaction:*

Oxazole rings may experience ring-opening reactions under some circumstances, which can result in the creation of open-chain compounds. The C-O or C-N link in the oxazole ring may be broken during this reaction.

### *3.4. Oxidation Reaction:*

Oxazole can undergo oxidation in the right circumstances. This could entail the ring's carbon or nitrogen atoms oxidizing. One may use common oxidizing agents such as permanganates or chromates.**[5]**

### *3.5. Reduction Reaction:*

Dihydrooxazole or tetrahydrooxazole derivatives can be produced when oxazole is reduced. For this, reducing agents like lithium aluminum hydride ($LiAlH_4$) can be employed.

### *3.6. Functional Group Transformation:*

Depending on the reaction circumstances and chemicals employed,

oxazole can undergo a variety of functional group transformations, including acylation, alkylation, and formylation

### *3.7. Condensation Reaction:*

By removing water or other smaller molecules from condensation events, azole can take part in the formation of bigger compounds.

Oxazole will react differently depending on the exact circumstances, reagents, and substituents that are in the molecule. Furthermore, oxazole is useful in synthetic chemistry since it may be used as a building block to create more complicated organic molecules, which is particularly useful in drug discovery and medicinal chemistry.**[6]**

## 4. BIOLOGICAL ACTIVITIES OF OXAZOLE

### *4.1. Oxazoles as Antibacterial Agents:*

In recent years, Enterococcus has emerged as a drug-resistant bacterium leading to an alarming increase in bacterial infections. *Enterococcus faecium*, of the ESKAPE pathogens category, are responsible for causing most bacterial infections. Narrow-spectrum antibiotics are developed when the enzyme D-aspartate ligase of *Enterococcus* is targeted. Reports suggest that oxazole analogs show good activity against *Porphyromonas gingivalis* and *Streptococcus gordonii* when 1,2,3 triazole moiety is introduced into its structure.

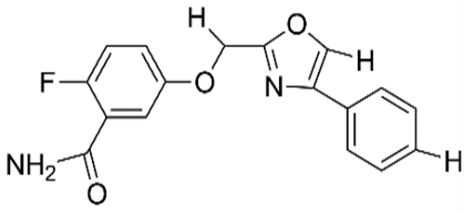


**Figure 4:** Oxazole moiety as an effective antibacterial agent.

Studies also suggest that oxazole compounds having *benzamide ring* in their structure possess good antibacterial activity against *Staphylococcus aureus* having MIC values from 0.06- f16microgm/ml as depicted in structure 6. Reports have shown that Isoxazoles which are isomers of oxazoles work as potent antibacterial agents. Reports also prove that oxazole compounds containing aryl sulfonate moiety show good antimicrobial activity. Zhang et al **[7]** showed that Isoxazole having a tosyloxy phenol group gave good results when used against Aeruginosa, and quinone, when attached to the Isoxazole structure, gave good activity against *Bacteria subtilis*.

### *4.2. Oxazole as an Antifungal Agent:*

The recent years have experienced new technologies have been used in the treatment of diseases to overcome the mortality rate but it has also resulted in the advent of other diseases mainly as a side effect of the treatment and therapies. Invasive fungal diseases among infectious diseases represent a remarkable proportion **[8].** Currently, around 300 million people are affected by fungal infections and it has caused the deaths of 1.6 million people annually **[9].** The global health impact of fungal infections includes high morbidity, 30–80% mortality, and a multi-billion annual financial burden **[10].** *Candida, Cryptococcus, Aspergillus,* or *Pneumocystis* are the most common species to which most of the fungal pathogens belong but in recent years, there are fungal species that have been reported from classes that are unknown or lesser known. *Rhodotorula* species are responsible for causing fungal infections like meningitis, fungemia, and peritonitis. *Cladosporium* species are related to allergic rhinitis, respiratory arrest in asthmatic patients, and phaeohyphomycotic conditions. The antifungal class consists of drugs belonging to the class of azoles such as Fluconazole, Ketoconazole, Clotrimazole, Itraconazole, polyenes having amphotericin B, nystatin, and

natamycin. In addition to these, terbinafine and naftifine belong to the class of Allylamines, Pyrimidine analogs consisting of 5-flourocytosine and 5-flourouracil along with caspofungin, anidulafungin, micafungin belonging to the class of echinocandins. Azoles are often used as first-line drugs because they can manage systemic and topical infections efficiently, are cost-effective, and target effectiveness to the majority of fungal infections. Research further shows that oxazoles can also work as antifungal agents and be as useful and potent as other azoles. The structure-activity relationship proves that when oxazole is introduced into *the indole* skeleton it gives good results against *Alternaria brassicicola* when used at a concentration of 0. 50Mm. Isoxazole showed good activity against antifungal activities when it was attached to drugs like Ravuconazole and replaced the original cyanophenyl thiazole moiety from ravuconazole. Isoxazole which is linked to Imidazolidine moiety is a potential pharmacophore that can be exploited for future studies as it exhibits good activity against fungal strains. Reports showed Their activity was further increased against fungal strains when they were substituted with halogen and methoxy groups.

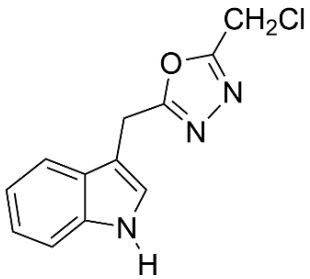


**Figure 5**: Oxazole fused with Indole

### *4.3. Oxazoles as Antiviral Agents:*

Hepatitis C and HIV are viral diseases that have existed in the world for a long time now, and still, they rely on therapies that have low success rates ultimately the virus becomes resistant to them resulting in high mortality rates across the world. Zhang et al. showed that the incorporation of an oxazole ring into pyrrolo-triazine resulted in a compound that has good activity against HCV RNA replication. This compound thus synthesized led to further synthesis of a nucleoside or nucleotide drug for use in future anti-HCV therapy. The biological activity of oxazoles was further enhanced when it was attached to heterocyclic rings such as thiazole, indole, pyridine, benzene, benzothiophene as they had good antiviral activity against HCV (Hepatitis C virus), and reduced cytotoxicity.

This further led to the conclusion that oxazole derivatives are the most promising candidates for antiviral activities and can be exploited as novel drug targets for viral diseases. Cell-based screening of an in-house library and subsequent scaffold modification led to the discovery of various oxazole compounds such as bis-substituted oxazole compounds.

Zhang et al. have further elaborated on the structure-activity relationships where it was seen that excellent anti-HIV-1 Inhibitory activity was shown with dimethoxy phenyl analogs. Studies also show that viruses were quite susceptible to the substituents when they were positioned in the 5$^{th}$ position of the *aryl ring* as shown in structure 8 and were tolerant when electron-withdrawing groups such as chloro and nitro groups were positioned at the 4$^{th}$ position.

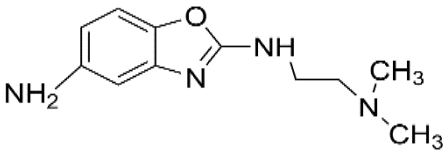


**Figure 6:** Oxazole having bis substitution.

### *4.4. Oxazoles as Anti-cancer Agents:*

Cancer-related deaths are majorly driven by metastasis which numerous cellular processes when they become irregular further making malignant cells leave their point of origin and spread themselves in distant sites leading to malignant tumours. Bis-substituted oxazole compounds showed excellent anti-cancer activity. Intense research on the structure-activity relationship reveals that for showing good therapeutic activity, aryl compounds such as naphthalene-2-yl and quinoline-3-yl are required at the 5$^{th}$ position of the oxazole ring. Oxazole analogs having moieties such as pyridazine and 3,5-difluorophenyl residues are far more active than the corresponding oxadiazole analogs. diphenyl oxazole derivatives were synthesized successfully as they showed significant in-vitro anticancer activities and had promising activity on the HepG2 cell line. Research further showed that Diphenyl oxazole derivatives serve as promising candidates as anti-cancer agents and can thus be exploited for further studies and research work.

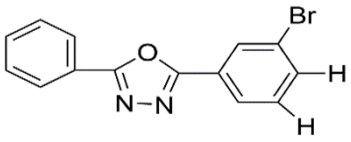


**Figure 7:** Oxazole having diphenyl substitution.

### *4.5. Oxazoles as Antitubercular Agents:*

Over the years, multiple drug-resistant strains of *Mycobacterium tuberculi*, have emerged which have caused a significant decrease in the treatment regimen. With the strains becoming resistant, efforts have been made to synthesize novel compounds that have good activity, less toxicity, and provide immunity against various strains of tuberculosis. Isoxazole derivatives have time and again proved their pharmacological importance by exhibiting various activities against microbes. ***Phenyl -Isoxazole derivatives*** when attached to an electron-donating isopropyl group showed significant activity against tuberculosis. SAR studies further revealed when the same isoxazole derivative was attached to methyl and methoxy groups, activity was significantly reduced.

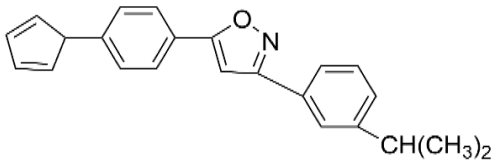


**Figure 8:** Phenyl Oxazole derivative having good activity against tuberculosis

## 6. CONCLUSION

To sum up, this review has given a thorough overview of the many facets of oxazole chemistry and its importance in a range of disciplines, such as materials science, natural product synthesis, and medicinal chemistry. Because of their distinct structural characteristics and varied reactivity, azoles have become useful building blocks with a broad range of applications. Researchers all over the world are still particularly interested in oxazoles because of their many applications, including as pharmacophores in drug development and as useful building blocks for creating new functional materials.

Additionally, improvements in synthetic techniques have made it easier to obtain a variety of oxazole derivatives, which has allowed for the investigation of their potential to solve a range of issues in materials science, medicine, and other fields. Furthermore, the fact that oxazole-containing compounds are found naturally in physiologically active molecules emphasizes the significance of these compounds as scaffolds for drug discovery and design.

## 7. SUMMARY AND FUTURE PROSPECTS

To sum up, this review clarifies the complex properties of oxazole and its present significance across a range of scientific domains. The potential of oxazole derivatives as various antimicrobials and anticancer agents is still being explored, and many novel approaches toward synthesizing oxazole have taken place in recent years. Materials science, catalysis, and advanced technologies have all proved beneficial in drug discovery and the synthesis of oxazole. Thus, as more and more work is put into the research and development of oxazole medications, a growing number of medications with good curative effects, low toxicity, and superior pharmacokinetic properties will be used in clinics, significantly advancing the prevention and preservation of human health.

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# From Labs to Communities: Practical Insights into Colistin Resistance Detection in Low-Resource Settings

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**A B S T R A C T**

Global healthcare systems face a significant challenge due to the prevalence of multidrug-resistant Gram-negative bacteria (MDR-GNB) in clinical samples. Colistin is frequently recommended for treating patients with multidrug-resistant bacteria. Developing effective screening methods to detect colistin resistance among Gram-negative bacteria in clinical samples is essential to prevent their rapid spread and subsequent patient consequences. There are several methods for detecting colistin resistance. The Clinical and Laboratory Standards Institute (CLSI) advised utilizing the broth microdilution (BMD) technique to test for colistin susceptibility till 2019. However, the CLSI has advocated the use of the colistin broth disc elution technique (CBDE) since 2020. The CBDE technique provides a very simple way of identifying colistin resistance, making it more appropriate for routine use. The CBDE and BMD methods are reliable and accurate methods for detecting colistin-resistant gram-negative bacteria. Thus, Colistin broth Disk Elution may be implemented in laboratories. However, a novel Colistin Disk Elution Screening method developed by Shubham et al. is cheaper than Colistin broth Disk Elution which can be used as an alternative in resource-limited settings.

Humanity has been combating diseases caused by microbial pathogens since ancient times. The 21st century is seeing increased global interest in microbial infections caused by drug-resistant pathogens. Due to the evolution of drug resistance among bacterial pathogens, multiple antibiotics become ineffective, ultimately resulting in limited and narrow treatment options. This global crisis poses a significant economic burden on healthcare systems and a threat to public health worldwide. The rise of multidrug-resistant bacteria is a natural consequence of bacterial evolution. Still, it has been accelerated by the rapid and extensive spread of Extended Spectrum Beta Lactamase (ESBL) within bacterial pathogens and resistance to carbapenem drugs. These bacteria pose a significant risk to the general public's health since they have steadily evolved resistance to most antibiotics, including penicillin and cephalosporins [1].

Global healthcare systems face a significant challenge due to the prevalence of multidrug-resistant Gram-negative bacteria (MDR-GNB) in clinical samples. Colistin is frequently recommended for treating patients with multidrug-resistant (MDR) bacteria, especially those exhibiting resistance to carbapenems. Colistin is considered to be a crucial antimicrobial agent of last resort [2]. By using the bacterium *Paenibacillus polymyxa* subspecies colistinus, Y. Koyama discovered the first "Colistin," also known as Polymyxins, in 1947. Colistin was

initially discovered but was then forgotten because of concerns about its conceivable nephrotoxic and neurotoxic characteristics [2].

In November 205, the first case of colistin resistance was documented; it posed a significant problem for the therapeutic community. Changes in the target site lipopolysaccharide (LPS), often resulting from chromosomal mutations, are the main source of colistin resistance. Furthermore, plasmids carrying the *mcr* gene aid in the transmissibility of this resistance. The World Health Organization (WHO) has classified colistin-resistant organisms and multidrug-resistant Gram-negative bacteria (MDR-GNB) as high-priority organisms due to the serious repercussions of infections brought on by these microbes. This classification considers the significant infection-related mortality rates that have been seen, requiring prompt action.[2,3,4]

Studying the pharmacokinetic (PK) /pharmacodynamic (PD) association of colistin in patients is important. Although data on the PD/PK of colistin are limited, particularly in the case of individuals with renal failure, it has been established that colistin concentration is critical for its antibacterial activity. A plasma concentration of colistin 2 g/L is considered a not harmful dose for microorganisms with 1 g/L MICs (minimum inhibitory concentration).[5]

Colistin resistance has been observed in gram-negative clinical isolates, including *Pseudomonas*, *Acinetobacter species*, and Family Enterobacteriaceae. In contrast, Proteus, Morganelland *Providencia* specieses naturally resist the antibiotic colistin.[6] However, evidence of colistin resistance is severely lacking in South Asian countries. Therefore, colistin resistance must be tested (particularly for *mcr* gene) in isolates obtained from clinical samples. Until 2015, when colistin resistance was first identified in a Chinese city, researchers believed that chromosomal genes, such as phoPQ, pmrAB, and mgrB, were the only sources of the resistance. However, 27 bacterial species from six continents were confirmed to carry the mcr-1 gene. With limited resources, all clinical diagnostic laboratories must standardize colistin testing methods so clinicians can use the drug more effectively to treat infections caused by MDR-GNB. [7,8]

Developing effective screening methods to detect colistin resistance among Gram-negative bacteria in clinical samples is essential to prevent their rapid spread and subsequent patient consequences. There are several methods for detecting colistin resistance, including the colistin broth disc elution method, CHROM agar, rapid polymyxin NP (P. Nordmann and L. Poirel) testing, the colistin agar technique, the ResaPolymyxin NP test, COL-APSE (colistin-resistant *Acinetobacter, Pseudomonas, Stenotrophomonas*, and Enterobacteriaceae), and the LBJMR method. It is crucial to develop and implement an efficient screening strategy to effectively identify colistin resistance and carry out the required therapies to stop the spread of these multidrug-resistant bacteria, ensuring patient health outcomes.[9-12]

The BMD (Broth Microdilution) method has gained recognition from both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standard Institute (CLSI) as a gold standard testing method.[12] It is essential to conduct the test using colistin sulfate salt in Mueller Hinton broth (cation-adjusted) without polysorbate-80 in a microtiter plate to obtain accurate results.[9,10] Due to the size of colistin molecules, the disc diffusion technique cannot be used to identify colistin resistance. Therefore, developing a cost-effective, precise, and easy-to-perform test to detect colistin resistance is crucial for laboratories with limited resources.

The Clinical and Laboratory Standards Institute (CLSI) advised utilizing the broth microdilution (BMD) technique to test for colistin

susceptibility till 2019. However, the CLSI has advocated of using the colistin broth disc elution technique (CBDE) since 2020. However, the BMD approach has limitations like time consumption that prevents it from being used frequently.[10,11] In contrast, the CBDE technique provides a very simple way of identifying colistin resistance, making it more appropriate for routine use. **(Figure-1)** However, a reliable approach needs to be developed to lessen the morbidity and mortality caused by colistin resistance. The Colistin broth Disk Elution method and broth microdilution method are reliable and accurate methods for detecting colistin-resistant gram-negative bacteria. Thus, Colistin broth Disk Elution may be implemented in laboratories. However, a novel Colistin Disk Elution Screening method developed by Shubham et al. in 2022 is cheaper than Colistin broth Disk Elution and can be used as an economical alternative in resource-limited settings.[13]


**Figure 1** Colistin broth elution method used to detect colistin resistance. (Test MIC - 4µ/ml)

(A) Growth Control; (B) 14µ/ml; (C) 2µ/ml (D) 4µ/ml

## CONCLUSION

Global healthcare systems face a significant challenge due to the prevalence of multidrug-resistant Gram-negative bacteria in clinical samples. Colistin is frequently recommended for treating patients with multidrug-resistant bacteria infections. Therefore, developing effective screening methods to detect colistin resistance among Gram-negative bacteria is essential. The CBDE technique provides a very simple way of identifying colistin resistance, making it more appropriate for routine use. However, a novel Colistin Disk Elution Screening method is more reliable and cost-effective than CBDE, and therefore, it can be used as an alternative in resource-limited settings.

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# A Review of The Trends in Drug Discovery Using Machine Learning and Artificial Intelligence

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**A B S T R A C T**
This is a concise overview of the trends in drug discovery facilitated by machine learning (ML) and artificial intelligence (AI). Examining advancements in predictive modeling, target identification, and compound optimization, the article highlights the integration of diverse data sources and the emergence of deep learning techniques. Challenges such as model validation and ethical considerations are addressed despite these strides. It underscores ML and AI's transformative potential in enhancing the efficiency of drug development processes.

**Keywords:** Artificial Intelligence, Drug Discovery, Machine Learning, Machine Learning algorithms, QSAR study.

## 1. INTRODUCTION

Developing a new medication is a challenging and costly process that can take up to 15 years **[1]**. Even the most skilled scientists face setbacks, which can result in significant financial losses. Despite advancements in technology and biological research, productivity in the pharmaceutical industry has decreased, leading to fewer newly approved drugs due to regulatory hurdles and difficulties in finding new treatments that outperform current options **[2]**. To address these challenges, researchers have turned to computational methodologies like virtual screening and molecular docking. However, these techniques can be inaccurate and inefficient **[3]**. To overcome these problems, novel approaches that are self-sufficient and more effective than traditional computational methods have emerged. Artificial intelligence, involving deep learning and machine learning algorithms, has proven to be a valuable tool in simplifying complex processes, resulting in significant time and cost savings **[4]**.

With the growing prevalence of digital data in the pharmaceutical and healthcare industries, AI technology has become increasingly necessary for data analysis. Artificial Intelligence (AI), also referred to as machine intelligence, refers to the ability of computer systems to learn from input and data from the past. The term AI is used to describe the process by which a machine imitates cognitive behaviours associated with the human brain during learning and problem-solving **[5]**. Machine learning (ML) possesses the capacity to expedite pharmaceutical research by uncovering new and significant insights from the extensive and intricate data produced during the drug discovery process. In recent times, AI/ML-driven approaches have been extensively employed across various therapeutic domains, demonstrating cutting-

edge effectiveness in tackling a variety of challenges within drug discovery.

Our aim is to review the historical background, overview of AI/ML in finding novel compounds, and its applications in collaboration with conventional chemistry for drug discovery. We will also discuss the challenges and potential future scope for this technology.

## 2. EVOLUTION OF AI/ML IN THE FIELD OF PHARMACEUTICAL DRUG DISCOVERY

Artificial Intelligence (AI) was first proposed by American scientist Professor John McCarthy in 1956. With the rise of computers and Graphical Processing Units (GPUs), scientists were able to incorporate human thinking and learning abilities into computers. This allowed computers to learn and implement new knowledge **[6]**. In recent decades, AI and machine learning techniques have been implemented in drug discovery, classification of active and inactive molecules from large datasets, QSAR studies, clinical trials, and providing precision medicine to patients based on their genetic disposition.

## 3. OVERVIEW OF AI/ML IN DRUG DISCOVERY

Based on Vatansever S. et.al's review of AI/ML based drug discovery in central nervous system diseases, 2021 **[7]**, AI/ML model learning techniques are classified as follows:

### *3.1. Supervised Learning:*

In supervised learning, the algorithm is trained on a labelled dataset, where each input is associated with a corresponding output. The goal is to learn a mapping from inputs to outputs, making it possible to make predictions or classify new, unseen data.

### *3.2. Unsupervised Learning:*

This deal with unlabelled data, aiming to find patterns, relationships, or structures within the data without explicit output labels. Common tasks include clustering, dimensionality reduction, and density estimation.

### *3.3. Semi-Supervised Learning:*

This is a combination of supervised and unsupervised learning. The model is trained on a dataset that contains both labelled and unlabelled examples. This approach is particularly useful when labelling data is expensive or time-consuming.

### *3.4. Active Learning:*

This is a strategy where the algorithm interacts with a human (or an oracle) to obtain labels for selected instances. The goal is to intelligently choose which instances to query for labels, with the aim of improving the model's performance with minimal labelling effort.

### *3.5. Reinforcement Learning:*

This involves training an agent to make sequences of decisions by interacting with an environment. The agent receives feedback in the form of rewards or penalties, allowing it to learn optimal strategies to achieve a goal or maximize cumulative reward over time.

### *3.6. Transfer Learning:*

This is a technique where a model trained on one task is adapted to a related but different task. The knowledge gained from the source task is leveraged to improve the performance of the model on the target task, especially when labelled data for the target task is limited.

### *3.7. Multitask Learning:*

This involves training a model to perform multiple tasks simultaneously. The idea is that the shared knowledge across tasks can improve the model's overall performance. It's particularly useful when tasks have some underlying commonalities.

### *3.8. Multiple Kernel Learning:*

This is a technique in machine learning where multiple kernels (functions measuring similarity between data points) are combined or weighted to form a composite kernel. This allows the model to consider different aspects or representations of the data, potentially improving performance over a single kernel.

### *3.9. Ensemble Learning:*

This involves training multiple models and combining their predictions to improve overall performance. The idea is that by combining diverse models, the ensemble can often achieve better results than individual models. Common methods include bagging (e.g., Random Forests) and boosting (e.g., AdaBoost, Gradient Boosting).

### *3.10. End-to-End Learning:*

This is an approach in machine learning where a model is trained to perform a task directly from raw input to the final output without explicit intermediate representations or manual feature engineering. The model learns to map input data to the desired output in a single, integrated process. This approach is often used in deep learning and neural networks.

## 4. AI/ML PROBLEMS

AI/ML problems are classified as follows:

- Regression- Regression is a type of supervised learning used to predict a continuous outcome. Regression models can be applied to predict properties of drug candidates, such as binding affinity or biological activity, based on various molecular features and descriptors.
- Classification- Classification is a type of supervised learning employed to categorize compounds into different classes or groups. It can be used to predict whether a molecule possesses certain desired properties, such as therapeutic efficacy or toxicity, allowing researchers to make informed decisions about potential drug candidates.

*The most trending algorithms that are utilized are:*

- Naïve Bayes- Simple probabilistic model, efficient with high-dimensional data, assumes feature independence.
- Support vector machines- Effective for high-dimensional spaces, optimal separation of classes, versatile kernel functions.
- Random Forest- Ensemble of decision trees, robust and accurate, handles complex relationships in data.
- K-nearest-neighbors- non-parametric, instance-based, proximity-based classification, suitable for local patterns.
- Artificial neural networks- Mimics biological neurons, adaptable to complex relationships, effective for nonlinear mappings.
- Deep neural networks- Hierarchical representation learning, suitable for large datasets, excels in capturing intricate patterns

## 5. MACHINE LEARNING METHODOLOGY IN DRUG DISCOVERY

The basic steps involved in machine learning methodology are:

1. Understanding of the problem which needs to be solved i.e., the target of interest.
2. Obtaining a dataset of compounds with established biological activity.
3. Converting the compounds into SMILES.
4. Generation of descriptors or fingerprints whichever is necessary.
5. Choosing machine learning algorithms.
6. Useful feature selection from the available descriptors.
7. Splitting the dataset into Training and Test sets.
8. Training of the ML model.
9. Internal validation of the selected ML algorithm with training set.
10. External validation of the selected ML algorithm with test set.
11. Finetuning the hyperparameters with cross-validation using the training set.
12. Model with good R2 value is then further used to get output for new dataset of molecules with gives fast and quick results.

## 6. APPLICATION OF AI AND MACHINE LEARNING IN DRUG DISCOVERY

### *6.1. Streamlining Drug Discovery:*

The vastness of chemical space, with over $10^{60}$ molecules, presents challenges in drug development due to time and cost constraints. However, leveraging AI technologies offers a promising solution by expediting the identification of potential drug candidates through advanced data analysis, predictive modelling, and simulation techniques, thereby streamlining the drug discovery process **[8, 9]**.

### *6.2. Reforming Drug Development:*

Enhanced computational power and advancements in AI offer promise for reforming drug discovery and development. The pharmaceutical industry currently grapples with declining efficiency in drug improvement programs and escalating research costs. Digitalization has inundated the industry with vast data, presenting a challenge in effectively utilizing this information for complex clinical problems. AI's capacity to handle extensive data through automation and employ machine learning algorithms to boost productivity is a focal point in this discussion, highlighting its potential to enhance the drug discovery cycle **[10, 11]**.

### *6.3. Predicting Physicochemical Properties:*

Cui and Zhu sought to assess AI's potential in predicting physicochemical properties **[12]** (solubility, partition coefficient, dissociation constant) of diverse drugs by employing a neural network known as ResNet. Their findings demonstrated enhanced accuracy in predicting molecule solubility compared to conventional non-AI models, resulting in increased polysaccharide yield and decreased extraction time from various sources. This highlights AI's capacity to augment drug development procedures, suggesting its integration could enhance overall efficiency in this domain.

### *6.4. Enhancing Drug Screening:*

Polykovskiy et al. **[13]** conducted a study to assess AI's efficacy in predicting the activity of synthesized molecules. Their aim was to enhance the reliability of drug screening by leveraging AI. Using an adversarial auto-

encoder, they predicted activity, generating compounds through de novo molecular design—yielding both random drug-like and target-biased compounds. Interestingly, the compounds obtained differed from those generated by a Recurrent Neural Network-based generative model, suggesting the complementary use of both approaches. This study not only highlighted AI's ability to improve procedural accuracy and efficiency but also unveiled valuable insights into molecule-target interactions, showcasing its capacity for facilitating novel discoveries.

### *6.5. Predicting Antimicrobial Properties:*

Daynac et al. **[14]** conducted a study to evaluate the efficacy of an artificial neural network in predicting the antimicrobial properties of diverse molecules, aiming to expedite, reduce costs, and enhance reliability in this process. Their findings revealed that the neural network achieved accurate predictions of over 70% of antimicrobial activity with a minimal error margin of 10 mm. Furthermore, the network demonstrated the capability to simultaneously forecast the behavior of two or three molecules, consequently reducing the overall processing time required for analysis.

### *6.6. Forecasting Toxicity Levels:*

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### *6.7. Hastening Anticancer Drug Screening:*

Two studies, Kadura et al. **[16]** and Maram and Hamdy **[17]**, employed distinct AI subcategories. Kadura et al. utilized adversarial auto-encoders and adversarial networks to hasten anticancer drug screening, evaluating approximately 72 million molecules and showcasing the technology's efficiency. Meanwhile, Al-Safarini and El-Sayed employed various AI processes which are used to forecast how molecules will behave upon interacting with various cell types, predicting their biological activity in those contexts. The studies collectively emphasize the diverse applicability of AI in the pharmaceutical domain, showcasing improved accuracy at 71.9% in contrast to the initial values of RF: 62.6% and SVM: 66.0%, with a negligible margin of error.

### *6.8. Machine Learning in GPCR Ligand Recognition:*

Raschka et al. in a study showcased machine learning integration in GPCR ligand recognition for drug discovery. They aimed to replace traditional technology in SLOR1 receptor signal inhibition experiments. The algorithm's results closely matched established standards, hinting at its potential to replace older methods for identifying molecule features. This study emphasized machine learning's adaptability across various sectors and its significant role in drug discovery **[18, 19]**.

### *6.9. Identifying Bitter Molecules in Early-stage Development:*

A study conducted by Margulis et al. **[20]** showcased the utilization of machine learning in drug discovery, focusing on the identification of intensely bitter molecules in early-stage development. The research aimed to

assess whether a specific machine learning algorithm could serve as a viable alternative to animal testing in predicting the bitterness of various drug-related compounds. Notably, around 80% of the identified bitter molecules aligned with outcomes from a brief access taste aversion (BATA) experiment, affirming the study's success. Furthermore, post-BATA experimentation revealed a disassociation between toxicity and bitterness, challenging prior assumptions. This underscores the efficacy of machine learning in providing accurate predictions while uncovering novel insights in drug development.

### *6.10. Analyzing Therapeutic Effects of Drugs on Genes:*

In a study assessing the potential of machine learning and deep neural networks in analyzing the therapeutic effects of various drugs on genes, significant advancements were observed. The integration of these technologies led to a substantial increase in the accuracy of predicting drug categories compared to baseline models. This improvement resulted in a more precise classification of drugs with diverse pharmacodynamic and pharmacokinetic properties. The enhanced accuracy underscores how combining deep learning neural networks with machine learning algorithms amplifies the effectiveness of drug classification systems **[21]**.

### *6.11. Showcasing Distinct Machine Learning Subsets:*

Rantanen and Khinast **[22]** along with Turki and Taguchi, **[23]** conducted studies showcasing distinct machine learning subsets for comparison. Turki and Taguchi utilized reinforcement learning to expedite drug identification, significantly shortening the process to a mere 46 days compared to conventional methods. Conversely, Zhavoronkov and Mamoshina applied transfer learning to predict multiple myeloma patient responses to a known drug, achieving improved predictive accuracy. Turki and Taguchi highlighted the need for algorithm refinement to diversify synthesized compounds, while Zhavoronkov and Mamoshina emphasized the necessity of expanding algorithmic scope, considering the data's limitations in broader applications beyond individual cases.

## 7. CONCLUSION

In summary, the integration of AI and machine learning into drug discovery has catalysed a paradigm shift in pharmaceutical research. These technologies have significantly expedited the identification of potential drug candidates and enhanced our ability to predict their effectiveness. Despite remarkable progress, challenges such as data reliability and regulatory compliance persist in translating these findings from computational models to tangible drug development. Collaborative efforts and ongoing technological advancements hold the promise of revolutionizing drug discovery, enabling more precise, efficient, and innovative approaches that have the potential to transform healthcare by delivering novel and effective therapeutics.

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# A Comprehensive Review of *In Silico* Modeling in Drug Design

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**A B S T R A C T**

*In silico* modeling has emerged as a very powerful and helpful tool in the field of drug design, revolutionizing the traditional approach to drug discovery. This computational method simulates and forecasts the behavior of molecules, interactions, and biological systems by utilizing sophisticated algorithms and computational methodologies. The identification and optimization of viable drug candidates has been greatly sped up by the incorporation of *in silico* modeling tools in drug design, which has decreased the length and expense of experimental trials. An overview of the main elements of *in silico* modeling for drug design is given in this article, which includes virtual screening, molecular docking, molecular dynamics simulations, and studies of the quantitative structure-activity relationship (QSAR). Researchers can thoroughly examine the complex terrain of molecular interactions, binding affinities, and structural dynamics within biological targets with the help of these approaches. This drug design strategies brought a new era of efficiency and creativity in pharmaceutical research has been brought about using *in silico* modeling in drug creation.

**Keywords:** *In Silico*, Drug design, Drug discovery, Docking, Molecular Modeling.

## 1. INTRODUCTION

*In silico* modeling plays a crucial role in modern drug design, revolutionizing traditional methods by employing computational techniques to analyze and predict the behavior of biological systems and drug molecules. The word *"in silico"* comes from the Latin *"in silicon,"* and it describes the method of simulating biological processes and interactions through computer simulations and algorithms, usually run on computers built on silicon.[1].

The introduction of *in silico* modelling in drug design has significantly expedited the drug discovery process, making it more cost-effective and efficient. This approach involves the use of various computational tools and techniques to understand the complex interactions between drugs and biological targets, predict pharmacokinetic properties, optimize drug candidates, and ultimately enhance the likelihood of successful drug development [1, 2].

## 2. TARGET IDENTIFICATION

A biological entity that can modify disease phenotypes—typically a protein—is referred to as a therapeutic target [5]. Therefore, the first and most crucial stage in the drug development process is identifying promising drug targets. Traditional methods for selecting therapeutic targets involve conducting experiments to find genes that are expressed differently in healthy tissues or cells impacted by an illness, as well as proteins that have strong connections to other proteins linked to the disease. [3, 4]

### *2.1. Trial methods:*

Traditional Trial methods of target recognition incorporate biochemical and molecular investigations of illness etiology. Even though these studies advance our understanding of a range of illnesses, they can be time-consuming in terms of locating possible treatment targets. Target discovery has been accelerated by

recent developments in genome-scale screening tools, including target deconvolution, haploinsufficiency profiling (HIP), and steady isotope labelling by amino acids in cell culture (SILAC).

The HIP assay is a thorough screening method intended to find possible therapeutic targets. It functions by increasing cellular sensitivity to substances and determining the gene products linked to tumour cell line survival throughout the whole genome [6]. The HIP assay's capacity to evaluate hundreds of genes at once without requiring prior knowledge of the underlying mechanisms behind the disease is one of its main advantages.

Target deconvolution is achieved by several methods, such as affinity chromatography, biochemical suppression, and protein microarrays. As a useful reverse screening method, SILAC makes it easier to identify proteins that interact with medicines and small molecule probes in an unbiased, comprehensive, and reliable manner. This technique has recently been combined with affinity chromatography and quantitative mass spectrometry-based proteomics to improve the accuracy of determining drug-protein interactions [7]. While there are some advantages to SILAC, several disadvantages prevent it from being widely and practically used. These disadvantages include (i) the high cost of isotope labelling; (ii) the need for sophisticated equipment like high-resolution mass spectrometers; and (iii) the laborious process of creating chemically immobilized drugs while maintaining their biological activity.

### *2.2. Computational Approaches for Target Identification:*

Due to their intricacy, experimental approaches are costly and frequently used on a small scale. Computational techniques have been developed to discover possible therapeutic targets to address these difficulties [8]. Target protein computational predictions can be obtained using protein networks, experimental data [9], or text mining-extracted literature. This strategy makes use of the notion that proteins with identical binding affinities may attach to ligands with equal structures, producing biological outcomes that are indistinguishable [10].

A multitude of algorithms, including machine learning and statistical models such as TarFisDock, TargetHunter, PharmMapper, and to predict the biological targets linked to a specific pharmaceutical compound, the Similarity Ensemble Approach was developed. When one does not know anything about pathophysiology beforehand, one common tactic is to use the ligand properties to locate protein targets. Lavecchia carried out a thorough analysis of several machine-learning models intended for ligand searches, employing fingerprints and molecular descriptors that symbolize the physical characteristics of a chemical compound (**Figure 1**).

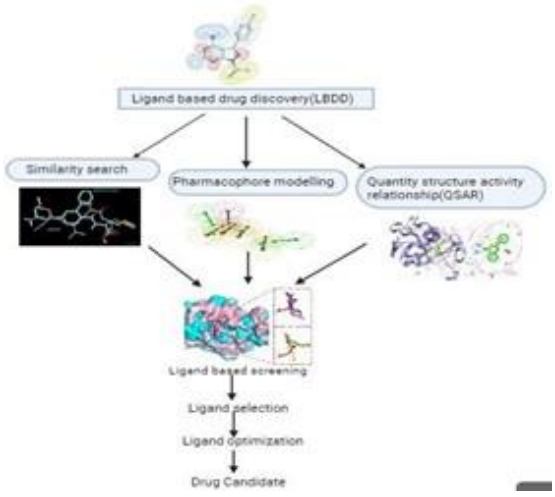


**Figure 1:** Drug discovery workflow based on ligands. Using a similarity search, pharmacophore modeling, or QSAR modeling, known active compounds are utilized to forecast new possible compounds from a vast number of chemical compounds. Next, to find new drug candidates, predicted compounds are put through biological property assessment and virtual lead optimization.

### *2.3. Verification of targets:*

Verifying if altering a target's biological function influences the illness phenotype comes

after a target has been discovered [11]. Modulating biological functions and assessing anticipated targets can be done in several ways. Small interfering RNAs (siRNAs) [12] are the most popular of these techniques because they can imitate the actions of drugs by suppressing translation, which causes the target protein to be temporarily suppressed. With siRNAs, target inhibition can be studied without the need for inhibitors or prior protein structural knowledge. In these circumstances, additional valuable information for target confirmation may come from animal prototypes where the desired gene has been changed or eliminated.

## 3. COMPUTATIONAL APPROACHES TO DRUG SCREENING

Finding tiny compounds that can alter the function of a particular target protein and change the illness phenotype is the aim of drug discovery. Finding tiny compounds with minimum toxicity and effective pharmacokinetic characteristics is also very important. The process of finding new drugs involves a long, expensive, and complex set of processes that include pharmacokinetics, preclinical toxicity assessments, candidate validation, and drug candidate identification.

Finding pharmacologically effective chemical compounds through screening is the main obstacle in drug discovery. In experimental High Throughput Screening (HTS), the hit rate typically ranges from 0.01% to 0.14%. Another major barrier is deficiencies in ADME-Tox (Absorption, Distribution, Metabolism, Excretion, and Toxicity), which accounts for 40–60% of medication failures in later phases. In the pharmaceutical industry, in computational drug discovery technology has long been a dominant force. Because it may be used at any stage of the drug development process, from drug testing to experimental and clinical phases, its primary benefits are time and financial efficiency. It also lowers the likelihood of failure considerably. [11].

### *3.1 Ligand-based drug screening:*

Ligand-based pharmacological Design (LBDD) techniques make use of the body of knowledge already available on active pharmaceuticals, including their structural, chemical, and physical characteristics, to forecast new pharmacological compounds that would have comparable biological effects. The forecast relies on assessing the degree of similarity among various chemical compounds based on characteristics such as aromaticity, hydrophobicity, and the existence of anion and cation residues. The fundamental idea is that substances with similar structures and physicochemical properties are more likely to possess comparable biological action. When the target protein's 3D structure is unknown, LBDD is frequently used. Target-ligand interactions can be better understood when the protein structure is unknown thanks to methods like pharmacophore modelling and Quantitative Structure-Activity Relationship (QSAR). Virtual screening of chemical compounds is made easier by a multitude of publicly available compound libraries.[10]

#### *3.1.1. Similarity Searches*

Finding novel compounds that resemble well-known active chemicals can be accomplished through the use of popular and efficient compound similarity searches. The underlying premise of these techniques is molecules having comparable physicochemical characteristics are more likely to possess comparable biological activity [11, 10]. A similarity search strategy has been used recently to identify several powerful chemicals; for instance, this approach has been used to generate agonists for a G-protein-coupled receptor (GPR30).

#### *3.1.2. Pharmacophore modelling*

For a chemical to interact with a protein target, pharmacophores have crucial electronic

and steric properties. Compound libraries are screened using pharmacophore models to find compounds with comparable structural and physicochemical properties. To find pharmacophores, different active ligands are scanned computationally for energetically stable conformations. The shared functional groups across the structures are then found by superimposing them [10, 11]. These pharmacophore-containing chemical compounds might be useful as novel therapeutic candidates. Pharmacophore modelling is done using a variety of programs, including PHASE, HipHop, HypoGen, and Ligand Scout. As demonstrated by the creation of novel inhibitors against the type II topoisomerase bacterial DNA gyrase B, which were derived from molecules in the ZINC database by pharmacophore modelling, this strategy has effectively assisted in the identification of more potent therapeutic compounds.

*3.1.3. Quantitative structure-activity relationships*

By using QSAR techniques, mathematical models are produced that link a compound's physical and structural characteristics to its biological function. Created in 1962 by Hansch and Fujita, QSAR is a well-known technique in drug development. Using this approach, QSAR models are trained using molecular descriptors that capture the structural and chemical features of compounds. The trained models are then utilized to predict the biological activity of specific chemicals, which can be used to find novel drug candidates or improve lead molecules. Chemical compounds having recognized biological activities are gathered for the creation of QSAR models, and these compounds are used for model validation and training. It is important to guarantee a structural variety of molecules to increase prediction accuracy [10].

***3. 2. Structure-Based Drug Discovery:***

It is a powerful approach in drug design that leverages the three-dimensional structures of biological macromolecules, typically proteins, to guide the identification and optimization of potential drug candidates. [1] This method relies on a detailed understanding of the atomic and molecular interactions between a target protein and small molecules, enabling the rational design of compounds with large affinity and specificity for the target. [1, 2] Instead of using ligand-based drug discovery methods, SBDD procedures use the structures of the target protein and ligand to establish the binding pocket—or affinity—between the two. This binding affinity prediction approach integrates molecular dynamic modelling, molecule docking, and fragment-based docking. [1, 2, 3].

*3. 2. 1. Target Protein Structure Generation*

In (SBDD), the most important step is to generate the 3D structure of a target protein**.** The structural information provides insights into the protein's binding sites and helps in designing small molecules that can interact with the target. [1, 4] Proteins with high sequence identity are assumed to have comparable arrangements and roles of 3D structures in homology modelling. For homology modelling, a wide range of tools are available; some of these are the Swiss Model, MODELER, Mod Web, and Phyre2. One option is to use ab initio protein structure modelling if there are no suitable template structures available. Small proteins (less than 100 amino acids) can be predicted using these techniques with an RMSD of 2.5–5 Å.

*3. 2. 2. Prediction of binding sites*

The concave area on a protein known as the binding site is where a ligand molecule attaches to provide the intended result (inhibition, modulation, or activation)**.** If the binding pockets' structural information is unavailable, potential binding pockets may be predicted using *in silico* methods.

*3. 2. 3. Molecular Docking*

A computational method called "molecular docking" is used in structure-based drug design to forecast the preferred orientation and binding affinity of a small molecule (ligand) in an active site of a target macromolecule, usually a protein**.** This method helps in understanding the interaction between a drug candidate and its target, allowing for the rational design and optimization of potential therapeutic compounds. The key steps involved in molecular docking are as 1. Preparation of Target and Ligand, 2. Grid Generation, 3. Search Algorithms, 4. Scoring Functions, 5. Analysis, 6. Validation and Refinement. There are several molecular docking software tools available, each with its algorithms, features, and capabilities. Molecular Docking Tools are Autodock, Autodock Vina, Gold, Glide, FlexX, Dock, DockIt, Lupi, HadDock, Fitted.

*3. 2. 4. Docking based on fragments*

It is a computational approach used in structure-based drug design, where the ligand (drug candidate) is assembled or docked into a target binding site in a stepwise manner using smaller fragments instead of docking the entire ligand at once. The initial stage in fragment-based docking is to create a structurally diverse library of fragments. Druggable fragments with a molecular weight of less than 300 Da, a cLogP of less than three, hydrogen bond donors of less than three, and hydrogen bond acceptors of less than three are typically created using the "rule of three". Subsequently, powerful segments are filtered according to their calculated binding affinity, just like in traditional molecular docking techniques. The screened segments usually have weak affinities because they contain important substructures like pharmacophores. To boost their efficacy, functional groups or extra pieces are added to the screened fragments.

*3. 2. 5. Molecular dynamic simulation*

It is a computational technique used to model the behavior of atoms and molecules over time. It provides insights into the dynamic aspects of molecular systems by numerically solving the classical equations of motion for each particle in the system. Molecular dynamics simulations are widely used in various fields, including chemistry, biochemistry, and material science, and they play a significant role in understanding the behavior of biological macromolecules, such as proteins and nucleic acids.

## 4. ADME-Tox ASSESSMENT

ADME-Tox assessment is a crucial aspect of drug discovery and development. Absorption, Distribution, Metabolism, Excretion, and Toxicity are referred to as ADME-Tox, and it is the process of assessing the safety and pharmacokinetic characteristics of putative treatment options. The following are important facets of ADME-Tox evaluation: 1. Toxicity; 2. Distribution; 3. Metabolism; 4. Excretion; 6. Pharmacodynamics; 7. Pharmacokinetics; 8. Bioavailability Predictions for ADME-tox, and 9. Regulatory compliance.

## 5. APPLICATION OF *IN SILICO* DRUG DESIGN

There are numerous uses for *in silico* drug design at different phases of the drug discovery and development process [1, 4]. Among the important applications are as follows:

- Target identification and validation.
- Lead identification and optimization
- ADME tox prediction
- Protein-ligand interaction studies
- De novo drug design
- Prediction of Drug-Drug interaction [1, 4].

## 6. SUMMARY AND FUTURE PROSPECTS

Using computational techniques to improve and expedite several phases of drug development,

*in silico* drug design has developed into a revolutionary strategy. Potential drug candidates are now identified, optimized, and validated using molecular docking, virtual screening, quantitative structure-activity relationship (QSAR) research, molecular dynamics simulations, and machine learning techniques. With the use of these computational techniques, scientists can analyze intricate chemical interactions, estimate binding affinities, and improve lead compounds—all of which greatly speed up the drug development process. Moreover, *in silico* methods aid in the prediction of pharmacokinetic characteristics, evaluation of toxicity, and investigation of polypharmacology, providing a comprehensive picture of a drug's possible effects. Treatments can now be customized depending on unique patient features thanks to the integration of proteomic and genetic data [8].

In conclusion, with the ongoing improvement of computational models, the incorporation of state-of-the-art technologies, and a cooperative, multidisciplinary approach, the future of *in silico* drug design holds tremendous opportunities. As these developments take place, *in silico* approaches have the potential to completely transform drug discovery and provide novel approaches to pressing global health issues [5, 8].

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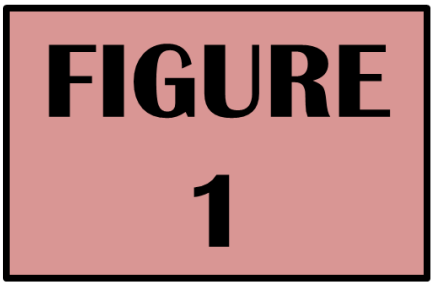


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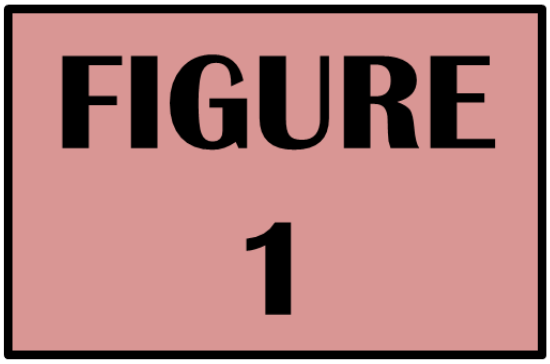


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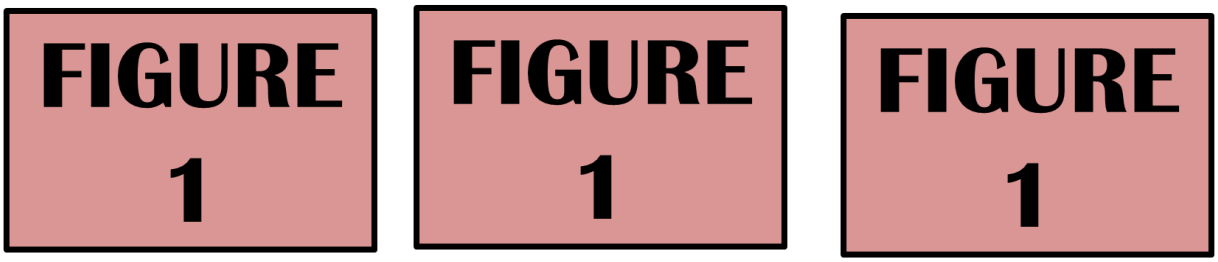


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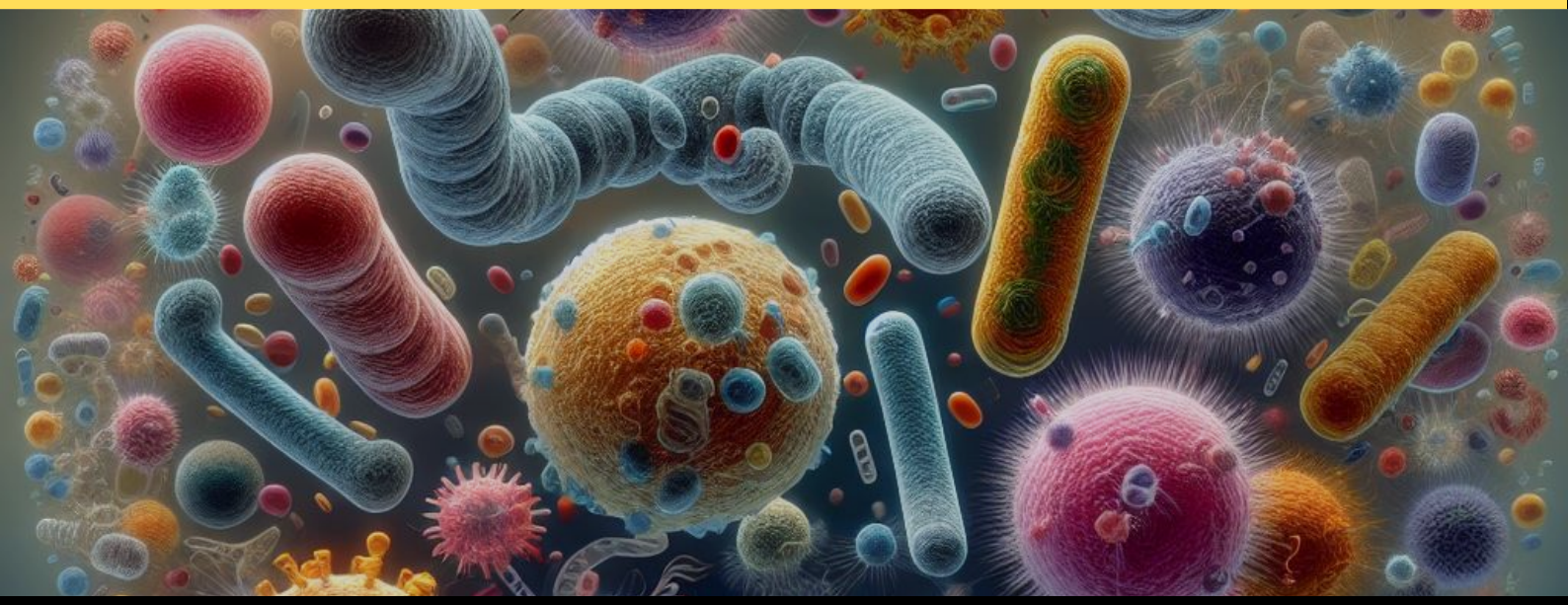

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