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A BRIEF ANALYSIS OF THE SIGNIFICANT PROGRESS IN THE SEARCH AND IMPLEMENTATION OF NEW DRUGS WITH HIGH ACTIVITY AND ONGOING SCIENTIFIC RESEARCH

Tolmas Khamroev Tolibovich[1]
Farangiz Erkinova Khasan qizi[2]
Sarvinoz Abdumalikova Akmal qizi[3]
Maryam Pardaeva Daminbek qizi[4]
[1]Department of pharmacology of Tashkent State Medical University
[2,3,4]Student of group 303A of the 3rd Faculty General Medicine of Tashkent State Medical University. e-mail: tolmas4th@mail.ru.
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ABSTRACT

Academic and industry scientists' methods for finding new pharmaceuticals have recently seen a real resurgence, with numerous innovative and fascinating approaches being created just in the last five to ten years. A significant obstacle is the ineffectiveness of current medications, the lack of information non society regarding the pharmacotherapy and prophylactics of the most prevalent diseases today, and the creation and discovery of novel, safe, and well-tolerated drugs. As the COVID-19 pandemic shown, this can affect not just the standard of living for individuals but also the health of entire societies. treatment discovery, preclinical development using animal and cell-based models and tests, human clinical trials, and, lastly, moving forward toward the step of getting regulatory permission, in order to sell the potential treatment, are the three key steps of the drug development process. As the success rate of drug development procedures rises, the pharmaceutical industry should be able to address current and future challenges and will continue to produce novel molecular entities (NMEs) to meet the growing unmet medical requirements of patients. Numerous heterocyclic moieties have been created, evaluated, and shown to be highly efficient against a variety of targets. This review's primary objective is to provide a thorough analysis of the drug design methodologies used for newly discovered medications between 2020 and 2022, including their pharmacokinetic and pharmacodynamic characteristics as well as in-vitro and in-vivo evaluations. This study is particularly relevant given the numerous phases these medications are undergoing in their clinical trials. Note

that synthetic strategies will be covered in a later publication and are not covered in this assessment.

Introduction. In order to thoroughly test and characterize the novel drug molecule for its pharmacological characteristics and toxicity profile, the process of drug discovery and development includes identification, optimization, pre-clinical and clinical research. An estimated 50% increase in OECD countries' expenditures on drug research and development has resulted from the successful completion of the Human Genome project in 2003, which produced a rough draft of the human genome. Several computational, experimental, and clinical models are used in the drug design and discovery process to identify possible novel therapeutic agents. Drug discovery is still a time-consuming, expensive, challenging, and occasionally ineffective process, even with advancements in biotechnology, pharmacology, and our comprehension of biological mechanisms [1-4]. Drug design begins by outlining substances that complement the molecular target they interact and bind to in terms of shape and structure. These days, computer modeling and bioinformatic methods are widely used in drug design. This implies that a synthetic method must already be in place or must be created in accordance with the physico-chemical characteristics of the reacting chemicals. When developing methodologies for the synthesis of target chemicals, consideration should be given to the environmental impact, production safety, economic perspective, and other green chemistry concepts. Drug discovery, pre-clinical development using animal and cell-based models and tests, human clinical trials, and, finally, advancing toward the step of obtaining regulatory approval in order to market the potential drug are the three main stages of the drug development process. The goal of contemporary drug discovery is to improve new medications' oral bioavailability, efficacy/potency, metabolic stability (to lengthen their half-life), selectivity (to lower the possibility of adverse effects), and affinity. Finding a hit molecule that produces the necessary activity in a screening test is the first step in the drug discovery process, as previously mentioned [5-11]. The hit molecule is next transformed into a lead molecule, or a therapeutic candidate, by optimizing its structure in order to increase its affinity and selectivity, decrease its toxicity, increase its water and lipid solubility, and improve its pharmacokinetic qualities generally. Subsequently, pre-clinical research aims to determine the drug candidate's mechanism of action and pharmacokinetics in animals (rodents, zebrafish), including its bioavailability, any hazardous metabolites, excretion routes, animal efficacy, drug formulation, and stability. Thirdly, clinical trials are the most costly and time-consuming step in the process.

They are conducted in three stages: first, on healthy volunteers; second, on a few hundred patients with the target disease; and third, involving thousands of patients from various clinical centers worldwide. This phase's objectives are to assess the drug's pharmacokinetics in the human body, its safety and effectiveness in people, and any immediate negative effects [12-18]. The medication is prepared for marketing and registration if it successfully completes this stage. Nonetheless, the medication's safety and adverse effects are still being monitored. This final stage, referred to as post-marketing surveillance, lasts for an almost infinite amount of time, until the medication is put on the market. Based on the experience of three collaborating and complementing academic centers of the Visegrád group, we will try to summarize the first three most significant successive stages in drug design and development in this review. Other evaluation efforts in this field may focus on the components of the legislative and regulatory laws intended to register a medicine and put it on the market. Due to the substantial amount of data gathered on this crucial subject, as well as the experiences of the authors and the worldwide project partners in this area of study, we specifically concentrated on small-molecule medications in our work [19-24]. There was also a brief discussion of several novel therapy approaches. In addition to using our own academic and scientific backgrounds from three complementing centers and collaborating scientists, we also planned to provide a quick overview of the design and development of a number of non-small-molecule medication types. Open talks for students, scientists, and the general public were held as part of the initiative to address various forms of these novel therapies [25-29].

The main purpose of this brief review is to analyze the significant progress made in the search for and implementation of new drugs with high activity, and ongoing scientific research based on reputable scientific papers.

Principles of Green Chemistry—A New Approach to the Synthesis of Drugs. Chemistry is all around us. It is used in the food industry, materials, electronics, cosmetics, and many other industries. Such a wide use of chemistry, however, can only be sustainable if we try to minimize its negative impact on the environment. With this aim, in the 1990s, Dr. John Warner and Dr. Paul Anastas developed the Twelve Principles of Green Chemistry. Green chemistry, by definition, is the design of chemical products and processes that reduce and/or eliminate the use or generation of hazardous substances. In order to reduce the risk that chemical reactions provide to the environment and human health, these principles are now modified in the great majority of chemical laboratories and considered when creating new substances. This also applies to the development and manufacturing of novel medications [27-32]. The catalysis of these reactions is one of the most intriguing aspects of green chemistry, which was swiftly and successfully used to the synthesis of organic molecules. Organic compound reactions frequently don't proceed with a 100% conversion of the starting materials; they can produce a lot of unwanted byproducts,

necessitate harsh conditions, etc. These issues (atom economy, decreasing derivatives, energy efficiency, etc.) are all incompatible with green chemistry. But there is a quite easy way to solve them: catalysis. The immobilization of catalysts on heterogeneous supports, such as polymers, oxides, and others, is one of the most popular and commonly used methods for obtaining highly recyclable, inexpensive, efficient, and selective catalysts. Enzymes, for instance, are now widely utilized in the production of fine chemicals, food, fragrances, tastes, medications, and other goods. In order to improve their effectiveness and other desired qualities, scientists have thus also concentrated on the best ways to immobilize them. There have been some intriguing new methods of immobilizing them revealed recently [13-21].

A succinct overview of drug discovery development and optimization, as well as the essential functions and significance of computer-aided drug design in this process. The goal of early-phase drug discovery is to identify highly promising synthetic compounds, semi-synthetic derivatives, or naturally occurring chemicals that exhibit a significant influence on different stages of a disease by advantageously regulating a specific biological signaling cascade or cascades. From the perspective of medicinal chemistry, this procedure is rather intricate, frequently takes a long time, and entails numerous theoretical and experimental studies as well as numerous optimization processes within certain areas of this scientific subject. Structure-activity, structure-pharmacokinetics, and/or structure-toxicity relationships are thoroughly investigated and correctly interpreted, and the platforms for optimization include the identification and appropriate characterization of pertinent biological targets, systematic screening, the highly accurate assessment of ligand (mono- or multi-functional drug)–target interactions, and logical drug design [12-17]. Determining the pharmacophore—a crucial component of the drug responsible for the intended biological activity—as well as appropriately selected bioisosteric alterations with the application of the principles and guidelines of bioisosterism constitute a key aspect of this optimization. In order to increase a biologically active compound's biological activity, selectivity toward a particular biological target or targets, metabolic stability, or, if selective toxic properties are not needed for therapeutic purposes, to reduce its toxicity, bioisosterism refers to the replacement of specific groups within the compound's structure. With respect to the intended biological activity and property-based design, other optimization techniques seek to appropriately alter the selectivity profiles of medications toward specific biological targets, including the structural, physicochemical, and pharmacokinetic (what the body does to the drug) characteristics of the medications or promising drug candidates [19-25]. Without a doubt, the several in silico methods described in this chapter will be used more frequently in early drug discovery to predict human pharmacokinetics. Pharmacodynamics, ADMET, and pharmacological characteristics for different sets of

substances may now be accurately predicted computationally. With a number of clear benefits, such as comparatively quick and dynamic evaluation procedures or the need for significantly fewer resources, such artificial tool outputs are most likely at least as good as those associated with in vitro research. Developing a well-balanced set of pertinent *in silico, in vitro,* and *in vivo* descriptors for simulating the drugs' pharmacodynamic characteristics and pharmacokinetics in particular human populations is the best method for contemporary drug design and development since it allows for the most accurate prediction of interindividual variability [30-35].

Numerous Advancements in In Vitro Drug Candidate or Drug Screening Methods. *In vitro* 2D culture screenings, *in vivo* evaluations, and human investigations are the three main categories into which the conventionally accepted methods for accurately testing medication candidates or pharmaceuticals can be broadly separated. High-throughput screening and bench-top and primary bioassay screening are two extremely strong subcategories of bioassay procedures. At every stage of the testing, the researchers make an effort to consistently use improved methodology and potentially novel experimental processes. Therefore, the next sections of the study provide a number of instances of such novel *in vitro* techniques. The so-called three Ms—determining a minimum inhibitory concentration value, determining efficacy using murine models, and assessing anti-TB activity in humans—are the driving forces behind the traditional lead optimization processes for anti-tuberculosis (anti-TB) medications during the pre-clinical stage of their development [6-12]. Using more logical and dynamic non-standard assays will help to better understand the locations of particular mycobacteria populations, which will optimize the in vitro screening of prospective anti-TB drugs and, consequently, the selection of anti-TB chemotherapeutic modalities. For carefully chosen and screened compounds, these compartments will serve as target sites. The broth-based in vitro composition, variations in pH and oxygen levels during specific infection phases, or oxygen availability must all be carefully considered by the researchers when merging in vitro settings into a single model. *In vitro* studies of a drug's or combination's pharmacodynamic/pharmacokinetic profile are made possible by hollow fiber devices, and the results are more in line with pharmacokinetic research conducted in vivo. According to evidence from *in vitro* and *in vivo* trials conducted under ideal settings, the application of appropriate combination therapy can result in the treatment of the disease in several bodily compartments [14-18]. This could lead to time and resource savings, a shorter duration of TB therapy, and a reduction in the quantity of anti-TB drugs needed to treat TB patients. A group of neurodegenerative illnesses known as human prion diseases are thought to be incurable. A prion protein that has misfolded is the cause of the illnesses. The absence of clear and well-established screening procedures has been one of the primary challenges in identifying reasonably acceptable

therapy options for humans. The use of a protein misfolding cyclic amplification procedure is one of the novel and efficient options for in vitro assays in the future. This technique can effectively replicate the pathological process of a particular physiological prion protein (PrPC) misfolding into an abnormally folded one (PrPSc) [19-24].

***In vitro* and *in vivo* preclinical research.** Serendipity, clinical observations, and animal studies were the mainstays of drug discovery in the past. These days, a novel chemical must pass a battery of exacting pharmacological and toxicological testing, known as "pre-clinical trials," before being authorized for human use in accordance with the logical order of research. In vitro research on genetically modified cell cultures is typically the first step to determine whether a medicine is carcinogenic or has an impact on reproduction. Biochemistry has been a significant contributor in recent years. Many medications used in human therapy have biochemical effects that can be attributed to their impact on particular enzymes or receptors [7-10]. The complexity of a single compound's action range increased as more and more receptor subtypes were discovered. Pharmacologists currently have access to human receptors and ion channels that are expressed in cultured mammalian cells thanks to molecular biology. Although the apparent species distinctions are avoided, the wide variety of natural and possibly man-made subtypes begs the question of their physiological and clinical significance. Molecular biology and biochemistry have contributed significantly in recent years. Many medications used in human therapy have biochemical effects that can be attributed to their impact on particular enzymes or receptors. Compared to traditional procedures, the employment of these techniques enables a considerably faster and more accurate characterization of the pharmacological action. They should only be carried out, though, if they are both required and well-thought-out [18-24]. The proper planning of the study, the choice of animal species, and the quantity and welfare of the animals all play a major role in the validity of the findings from pre-clinical *in vivo* investigations. Results from well-designed experiments can be extrapolated to people. Research on two distinct species, one of which is not a rodent, is appropriate. Because they are inexpensive, zebrafish (Danio rerio) and tiny rodents (mice and rats) are thus frequently used in our labs for study. If a pharmacological model's effects are consistent with those seen in human therapy, it can be deemed relevant or correlational. A model must be sensitive in a dose-dependent manner to standard compounds known to offer the desired therapeutic property in order to be considered significant or "correlated." Additionally, the model must be selective, meaning that the effects of the known agents in this therapeutic indication can be distinguished from the effects of drugs for other indications. Finally, the relative potency of the known active agents in the model must be comparable to their relative potency in clinical use. The prediction of a therapeutic impact in patients is made possible by positive findings with a novel drug [29-35].

General Information on Clinical Trials. When pre-clinical research yields encouraging results, the medication might proceed to clinical trials, which are conducted on human subjects. Clinical studies are conducted to evaluate the pharmacological and/or pharmacodynamic effects of the chemical molecule under test as well as to identify any adverse events (AEs). The International Council for Harmonization's (ICH) Good Clinical Practice (GCP) guidelines are followed when conducting clinical studies. GCP is a collection of ethical and legal guidelines that precisely outline how clinical trials should be planned, carried out, monitored, recorded, and reported. An independent bioethical committee must provide an opinion on the project during the clinical trial planning phase, and every stage of the study must be conducted with the highest care for the patient's welfare. The clinical research protocol, which outlines the trial's structure and progression as well as all the procedures that must be carried out as part of it, is the most important document in clinical trials [23-28]. The Informed Consent Form (ICF), the second most important trial document, must be signed by a patient (referred to as a study participant in a clinical trial) who voluntarily enrolls in the trial. It outlines the trial participant's rights and obligations, as well as the risks and benefits of the trial. The new medication (also known as the study drug or experimental product) may, but need not, aid in reducing the disease's symptoms. It is important to note that the research participant enters the clinical trial voluntarily. As a result, the study participant is free to leave the study at any time and for any reason. The majority of clinical trials are randomized, meaning that research participants are assigned to treatment groups at random. Each participant has an equal chance of being placed in a cohort that is receiving a study drug or a placebo. Both the patient and the doctor (known as the "Investigator" in clinical trials) are aware of the substance being provided in open-label clinical studies, although this could compromise the validity of the findings. As a result, the majority of studies are double-blind, which effectively implies that neither the researcher nor the study participant are aware of the therapy group to which they have been assigned. Clinical studies are divided into five primary stages [29-35].

Discussion. Drug research and discovery is a complex, diverse process. Preclinical research and clinical trials are the latter stages of this complex process, which starts with target discovery and validation and moves via chemical screening and lead optimization. This procedure begins with the identification of biological targets and pathophysiological variables. To identify cellular and genetic targets, bioinformatics, genomics, and proteomic research are required. First, the hit—the first molecule—that shows activity against the designated target is found. Chemical libraries or the isolation of natural compounds from fungi, plants, and bacteria can be used to accomplish this. The next stage involves identifying the lead chemical with the best chance of becoming a medication. The process of further altering the chosen lead to improve its specificity and efficacy, even

at lower dosages, is known as lead optimization [4-12]. Through an iterative cycle that combines structure–activity correlations and cellular assays, the functional features of recently synthesized therapeutic candidates are improved. Following that, the animal models are employed for in vivo research, such as toxicity evaluations and pharmacokinetic analysis. Following thorough preclinical testing, the medication candidate is eventually given to patients in clinical trials. In order to ascertain whether a drug candidate can provide the desired medical advantages while maintaining the patients' wellbeing, clinical trials are essential. The procedure is laborious and time-consuming. Pre-clinical and clinical studies are carried out as part of the drug research and development process in order to thoroughly assess and characterize the novel drug molecule for its pharmacological properties and toxicity profile. The cost of medication development is rapidly increasing, and it takes an average of 11.4 to 13.5 years for all drug development processes to complete several regulatory reviews. However, the increasing demand for new drugs is mostly responsible for the advancement in the development of more recent revolutionary medicines. Computer-aided drug design (CADD), which has significantly reduced the time required for the drug to go through the drug discovery phases, is one example of how techniques to find hits and lead compounds together with pre-clinical investigations have improved [14-21]. As the success rate of drug development procedures rises, the pharmaceutical industry should be able to address current and future challenges and will continue to produce novel molecular entities (NMEs) to meet the growing unmet medical requirements of patients. Numerous heterocyclic moieties have been created, evaluated, and shown to be highly efficient against a variety of targets. This review's primary objective is to provide a thorough analysis of the drug design methodologies used for newly discovered medications between 2020 and 2022, including their pharmacokinetic and pharmacodynamic characteristics as well as in-vitro and in-vivo evaluations. This study is particularly relevant given the numerous phases these medications are undergoing in their clinical trials. Note that synthetic strategies will be covered in a later publication and are not covered in this assessment. Novel mechanisms and a more younger patient population are driving our pharmaceutical industry forward, posing new hurdles like complex clinical trial designs that may result in lengthier and more costly drug development. Complementary diagnostic and screening tests may be useful in determining the target group and in cutting down on process time and expense. Last but not least, newly developed medications need to be efficient and reasonably priced, available to the general population, and differentiating from any current standards of care [24-31].

Conclusion. One of the aims of the medical sciences is drug research on novel, possibly useful medications, such as vaccinations, which is a crucial component of national and international health assistance initiatives. It takes more than 12 years to produce a new

medication, from molecular synthesis to target identification and marketing approval, according to analyses conducted across all therapeutic domains. Even with biotechnology advancements, this procedure is challenging, expensive, and time-consuming. Every scientific and biotechnological advancement has a direct impact on drug research and discovery, medicine, and pharmacy.

The better a drug candidate is designed in the experimental stage, the less likely it is to fail in the later stages, where tests are more costly, particularly in clinical trials, and the safer and more effective the drug will be. This makes investments in drug design worthwhile. For instance, the COVID-19 pandemic prompted researchers to reconsider how to shorten the timeframes for medicine and vaccine development. Drug discovery requires modern, efficient, and less expensive techniques, such as artificial intelligence, which can quickly collect and evaluate vast amounts of data, choose suitable targets and complementary ligands, and create and run experiments.

The ability to create and construct a specific, non-toxic, effective, and patient-tailored medicine over a number of hours is the ultimate goal for future drug development. In the near future, this objective is entirely attainable, despite the fact that it currently appears unrealistic. The increasing success rate of drug discovery procedures gives optimism that the pharmaceutical industry will be able to handle present and upcoming difficulties and will keep creating new medications and making them available to those in need.

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