REVIEW: AN UPDATE ON DRUG DISCOVERY AND NATURAL PRODUCTS

Ramesh S. Yamgar and Sudhir S. Sawant*

P. G. Dept of Chemistry, Govt. of Maharashtra’s Ismail Yusuf College of Arts, Science and Commerce, Jogeshwari (East), Mumbai 400060, India.

ABSTRACT
Nature is a source of diverse chemical structures useful as drugs to human being. This article deals with brief history of drug development since ancient times and evolution of drug products. It describes the development of drugs from natural sources in various therapeutic categories. Modern drug discovery approaches are mentioned with different stages using natural sources such as plants, microbial metabolites, marine invertebrates & animal sources. Transition metal complexes were prepared using coumarin, thiazole & benzophenone cores and screened against M. Tuberculosis.

Key words: History of drug development, Medicinal chemistry, Lipinski’s rule.

INTRODUCTION
The success story of the clinical uses of cisplatin, cis-[Pt(NH₃)₂Cl₂] and carboplatin has stimulated considerable interest in using other transition metal complexes as new therapeutic agents [1]. This perspective lead us to think to explore further research work on several classes of transition metal complexes for treatment of anti-cancer, anti-HIV treatments and various other therapeutic biological models. Iron-containing haemoglobin as a dioxygen carrier, calcium compounds used as basic constituents of bone, and template Zn²⁺ ions in three-dimensional structural frameworks of proteins are emblematic examples delineating the important roles of metal complexes in biological systems [2]. Apart from these endeavors, the unique properties of metal ions, such as redox transfer/electron shuttling, and versatile coordination geometries arising from various oxidation states, result in metal ions and their complexes having potential medicinal applications that could be complementary to organic compounds, the latter are widely sought in drug discovery efforts [3].

The uses of metal complexes as therapeutic agents can be traced back to 3500 BC [4]. Almost 5000 years ago, copper was used by the Egyptians to sterilize water. Over the past several decades, various antimony complexes were used for treatment of protozoan diseases like leishmaniasis and Trypanosomiasis. Medicinal inorganic chemistry as a discipline, however, started to develop after the serendipitous discovery of the anti-tumor activity of cisplatin [5]. The success of the clinical applications of this platinum complex has stimulated considerable interest in searching for new metal complexes as modern therapeutics, diagnostic and radiopharmaceutical agents, for example, silver(I) complexes commonly used as anti-microbial agents, bismuth(III) complexes for anti-ulcer treatments, gold(I) complexes as anti-arthritic agents, gadolinium(III), manganese(II) and iron(III) complexes as magnetic resonance imaging (MRI) contrast agents, technetium (⁹⁹Tc) and scandium (⁴¹Sc) as radiopharmaceutical agents [6]. In view of the emergence of drug-resistant cancer/viral strains and some undesirable side effects of cisplatin, there have been extensive studies from many laboratories worldwide to develop new metal based drug leads that could overcome the drug resistance and with fewer side effects[7]. As reported in recent reviews by Sadler and Lippard, there has been a growing interest in the chemistry community to examine the anti-cancer activities of gold(I, III), platinum(II), ruthenium(II, III), iron(II) complexes and the antiviral activities of vanadium(IV) complexes, some of these metal complexes have been developed to the stage of entering clinical trials [8].

Tuberculosis, caused by Mycobacterium tuberculosis, is one among the major infectious diseases and leading cause of mortality globally. Two million people die each year worldwide of which half a million are from India. The emergence of multi-drug resistant (MDR)TB, extensively drug-resistant (XDR) TB and HIV co-infection have exacerbated the global scenario of the disease.
The development of new drugs that can act against MDR and XDR TB and / or the one that will shorten the chemotherapy are the priority in TB research.

Apart from these applications, transition metal complexes are also explored in other therapies to treat poverty related diseases like TB/tuberculosis, typanosomiasis, malaria, dengue and filarial. Recent outbreaks of malaria, reemergence of chickungunya, enteric fevers, hepatitis and zoonotic diseases like brucellosis, ehrlichiosis, leptospirosis, anthrax etc pose serious health issues. Inhabitants in temperate zone may be exposed to danger of the infectious diseases owing to global warming [9]. In addition, the appearance and rapid spread of drug-resistant parasites reduced the effectiveness of conventional medicines and therefore, newer effective medicines are required by mankind to overcome the ever increasing health challenges. Genetic mutations in micro organisms and drug resistant infections like MDR and XDR TB, pose global threat. Hence there is an urgent need for newer, more effective, less toxic drugs, affordable medicines and drugs against resistant infections and unstoppable cancers.

New Drug Discovery for treatment of infectious diseases and cancer which could provide ultimate cure is an important area of research. Although cost prohibitive (~USD 500bn) with a meager success rate (~1%) the search for an ideal drug which could revolutionize therapy of infectious diseases and cancer continues unabated.

Transitional metal in combination with organic compounds lead to various possibilities towards developing cost effective and safe medicines for various therapeutic categories. It is well known that Schiff bases form coordination complexes with transition metal to get stable compounds having enhanced biological activities. Heterocyclic compounds having nitrogen atom incorporated in carbocyclic ring system possesses excellent biological activity due to in built pharmacophore.

History of Drug discovery and development

Drug discovery and development has a long history and dates back to the early days of human civilization. In ancient times, drugs were not only used for physical remedies but were also associated with religious and spiritual healing. The folk medicines were mainly derived from plant products, and supplemented by animal materials and minerals. Sages or religious leaders were often the administrators of folk medicines or early drugs. These drugs were most probably discovered through a combination of trial and error experimentation and observation of human and animal reactions as a result of ingesting such products [10].

Drug discovery and development started to follow scientific techniques in the late 1800s. From then on, more and more drugs were discovered, tested and synthesized in large-scale manufacturing plants, as opposed to the extraction of drug products from natural sources in relatively small batch quantities. After World War I, the modern pharmaceutical industry came into being, and drug discovery and development following scientific principles was firmly established [11-15]. The following are some snapshot examples of how drugs were discovered from the early human civilizations [16-18].

Chinese medicine

Traditional Chinese medicine is thought to have originated during the times of the legendary emperor Sheng Nong in 3500 BC. The dynasty system and meticulous recording have helped to preserve the scripts of old China. Some important medical writings are Shang Hun Lan (Discussion of Fevers), Huang Di Nei Jing (The Internal Book of Emperor Huang) and Sheng Nong Ben Cao Jing (The Pharmacopoeia of Sheng Nong-a legendary emperor). The Chinese pharmacopoeia is extensive. Some of the active ingredients from Chinese herbs have been used in ‘Western’ drugs; for example, reserpine from Rauwououfia for antihypertensive and emotional and mental control, and the alkaloid epihedrine from Mahuang for the treatment of asthma.

Egyptian medicine

Ancient papyrus provided written records of early Egyptian medical knowledge. The Ebers papyrus (from around 3000 BC) provided 877 prescriptions and recipes for internal medicine, eye and skin problems, and gynecology. Another record, from the Kahun papyrus of around 1800 BC, detailed treatments for gynecological problems. Medications were based mainly on herbal products such as myrrh, frankincense, castor oil, fennel, sienna, thyme, linseed, aloe and garlic.

Indian medicine

The Indian folk medicine, called Ayurvedic medicine, can be traced back 3000-5000 years, and was practiced by the Brahmin sages of ancient times. The treatments were set out in sacred writings called Vedas. The material medica are extensive and most are based on herbal formulations. Some of the herbs have appeared in Western medicines, such as cardamom and cinnamon. Susruta, a physician in the 4th century AD, described the use of henbane as antivenom for snakebites.

Greek medicine

Some of the Greek medical ideas were derived from the Egyptians, Babylonians, and even the Chinese and Indians. Castor oil was prescribed as a laxative; linseed or flex seed were used as a soothing emollient, laxative and antitussive. Other treatments include fennel plant for relief of intestinal colic and gas, and asafetida gum resin as an antispasmodic. The greatest Greek contribution to the medical field is perhaps to dispel the notion that diseases are due to supernatural causes or spells. The Greeks established that diseases result from natural causes. Hippocrates, the
father of medicine, at about 400 BC is credited with laying down the ethics for physicians.

**Roman medicine**

As great administrators, the Romans instituted hospitals, although these were used mainly to cater for the needs of the military. Through this work, organized medical care was made available. The Romans also extended the pharmacy practice of the Greeks. Dioscorides and Galen were two noted physicians in Roman days. Dioscorides’ *Materia Medica* contains descriptions of treatments based on 80% plant, 10% animal and 10% mineral products.

**DRUG DISCOVERY AND DEVELOPMENT IN THE MIDDLE AGES**

The Middle Ages, from around AD 400 to 1500, witnessed the decline of the Roman influences. This was also the time when plagues scoured many parts of Europe. Diseases such as bubonic plague, leprosy, smallpox, tuberculosis and scabies were rampant. Many millions of people succumbed to these diseases.

**The early Church**

There are some references to herbs in the Bible. However, the Church’s main contribution to medicines is the preservation and transcription of Greek medical manuscripts and treatises. This enabled the knowledge developed in the ancient times to be continued and later used in the Renaissance period.

**Arabian medicine**

Through trades with many regions, the Arabians learned and extended medical knowledge. Their major contribution is perhaps the knowledge of medical preparations and distillation methods, although the techniques were probably derived from the practices of alchemists. Avicenna, around AD 900-1000, recorded a vast encyclopedia of medical description and treatment. Rhazes was a noted physician, who accurately described measles and smallpox.

**FOUNDATION OF CURRENT DRUG DISCOVERY AND DEVELOPMENT**

The Renaissance period laid the foundation for scientific thoughts in medicinal preparations and medical treatments. There were many advances made in anatomy, physiology, surgery and medical treatments, including public health care, hygiene and sanitation.

In 1796, Edward Jenner successfully experimented with smallpox inoculations. This paved the way for the use of vaccination against some infectious diseases. In the late 1700s, William Withering introduced digitalis, an extract from the plant foxglove, for treatment of cardiac problems. John Hunter (1768) noted that scurvy was caused by the lack of vitamin C. He prescribed the consumption of lemon juice to treat scurvy. Louis Pasteur (1864) discovered that microorganisms cause diseases, and he devised vaccination against rabies. This was achieved through the use of attenuated rabies virus.

**BEGINNINGS OF MODERN PHARMACEUTICAL INDUSTRY**

Despite the advances made in the 1800s, there were only a few drugs available for treating diseases at the beginning of the 1900s. These were:

- **Digitalis**: extracted from a plant called foxglove, digitalis stimulates the cardiac muscles, and was used to treat cardiac conditions
- **Quinine**: derived from the bark of the Cinchona tree, and used to treat malaria
- **Ipecacuanha**: extracted from the bark or root of the Cephaelis plant, and used to treat dysentery
- **Aspirin**: extracted from bark of willow tree, and used for the treatment of fever
- **Mercury**: used to treat syphilis.

More systematic research to discover new drugs begun from the early 1900s. Paul Ehrlich used an arsenic compound, arsphenamine, to treat syphilis. Gerhard Domagh found that the red dye Prontosil was active against streptococcal bacteria. Later, French scientists isolated the active compound to be sulfanilamide, and this gave rise to a new range of sulfa drugs against hosts of bacteria.

**Penicillin**

In 1928, Alexander Fleming discovered that *Penicillium* mould was active against staphylococcus bacteria. Ernst Chain rediscovered this fact some 10 years later, when he collaborated with Howard Florey. By 1944, large-scale production of penicillin was available through the work of Howard Florey and Ernst Chain. This work foreshadowed the commencement of biotechnology, where microorganisms were used to produce drug products.

**EVOLUTION OF DRUG PRODUCTS [13-15]**

In the early days, until the late 1800s, most drugs were based on herbs or extraction of ingredients from botanical sources. The synthetic drugs using chemical methods were reported at the beginning of the 1900s, and the pharmaceutical industry was founded. Many drugs were researched and manufactured, but mostly they were used for therapeutic purposes rather than completely curing the diseases. From the early 1930s, drug discovery concentrated on screening natural products and isolating the active ingredients for treating diseases. The active ingredients are normally the synthetic version of the natural products. These synthetic versions, called new chemical entities (NCEs) have to go through many iterations and tests to ensure they are safe, potent and effective for the patients.

In the late 1970s, development of recombinant DNA products utilizing knowledge of cellular and molecular biology commenced. The biotechnology industry
became a reality. The pharmaceutical industry, together with the advances in gene therapy and understanding of mechanisms of causes of diseases, and the research results from the Human Genome Project, have opened up a plethora of opportunities and made possible the development and use of drugs specifically targeting the sites where diseases are caused.

Modern Drug Discovery and Natural Product Research

The World Health Organization estimates that approximately 80 percent of the world’s population relies primarily on traditional medicines as sources for their primary health care [20]. Over 100 chemical substances that are considered to be important drugs that are either currently in use or have been widely used in one or more countries in the world have been derived from a little under 100 different plants. Approximately 75% of these substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine [16,17]. The number of medicinal herbs used in China in 1979 has been estimated to be numbered at 5267 [25,26]. More current statistics based on prescription data from 1993 in the United States show that over 50% of the most prescribed drugs had a natural product either as the drug or as the starting point in the synthesis or design of the actual end chemical substance [27]. Approximately 39% of the 520 new drugs approved during the period 1983 through 1994 were either natural products or derivatives of natural products [22].

Indeed, if one looks at new drugs from an indication perspective over the same period of time, over 60% of antibacterials and antineoplastics were again either natural products themselves or based on structures of natural products. Of the 20 top-selling drugs on the market in the year 2000 that are not proteins, 7 of these were either derived from natural products or developed from leads generated from natural products. This select group of drugs generates over 20 billion U.S. dollars of revenue on an annual basis [21,22]. Drug development over the years has relied only on a small number of molecular prototypes to produce new medicines [22]. Indeed, only approximately 250 discrete chemical structure prototypes have been used up to 1995, but most of these chemical platforms have been derived from natural sources. While recombinant proteins and peptides are gaining market share, low-molecular-weight compounds still remain the predominant pharmacologic choice for therapeutic intervention [21]. Just a small sampling of the many available examples of the commercialization of modern drugs from natural products along with their year of introduction, indication, and company are: (Table 1).

The overwhelming concern today in the pharmaceutical industry is to improve the ability to find new drugs and to accelerate the speed with which new drugs are discovered and developed. This will only be successfully accomplished if the procedures for drug target elucidation and lead compound identification and optimization are themselves optimized. Analysis of the human genome will provide access to a myriad number of potential targets that will need to be evaluated [21,22]. The process of high-throughput screening enables the testing of increased numbers of targets and samples to the extent that approximately 100,000 assay points per day are able to be generated. However, the ability to accelerate the identification of pertinent lead compounds will only be achieved with the implementation of new ideas to generate varieties of structurally diverse test samples [21,22,23]. Experience has persistently and repeatedly demonstrated that nature has evolved over thousands of years a diverse chemical library of compounds that are not accessible by commonly recognized and frequently used synthetic approaches. Natural products have revealed the ways to new therapeutic approaches, contributed to the understanding of numerous biochemical pathways and have established their worth as valuable tools in biological chemistry and molecular and cellular biology. A few examples of some natural products that are currently being evaluated as potential drugs are mentioned in following table 2 [21].

For the period 1983 to 1994, seven out of 10 synthetic agents approved by the Food and Drug Administration (FDA) for use as antivirals were based on a natural product. These drugs are famciclovir, stavudine, zidovudine, zalcitabine, ganciclovir, sorivudine, and didanosine.

The costs of drug discovery and drug development continue to increase at astronomical rates, yet despite these expenditures, there is a decrease in the number of new medicines introduced into the world market. Despite the successes that have been achieved over the years with natural products, the interest in natural products as a platform for drug discovery has waxed and waned in popularity with various pharmaceutical companies. Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads. This is because the degree of chemical diversity found in natural products is broader than that from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source [18,22,28]. Where are these opportunities? Well, research into the use of plant derived natural products alone in just the field of medicine covers a broad spectrum of activities [19,24,29,30]. Examples of such biological activity profiles would include, but are not limited to, nootropics, psychoactive agents, dependence attenuators, anticonvulsants, sedatives, analgesics, anti-inflammatory agents, antipyretics, neurotransmission modulators, autonomic activity modulators, autacoid activity modulators, anticoagulants, hyloipidemias, anhypertensive agents, cardioprotectants, positive ionotropes, antitussives, antiasthmatics, pulmonary function enhancers, antiallergens, hypoglycemic agents, antifertility agents, fertility-enhancing agents, wound healing agents, dermal healing agents, bone healing agents, compounds useful in the prevention of urinary calculi as well as their...
dissolution, gastrointestinal motility modulators, gastric ulcer protectants, immunomodulators, hepato-protective agents, myelo-protective agents, pancreato-protective agents, oculo-protective agents, membrane stabilizers, hemato-protective agents, antioxidants, agents protective against oxidative stress, antineoplastics, antimicrobials, antifungal agents, antiproteozal agents, antihelmintics, and nutraceuticals [19]. Many frontiers remain within the field of natural products that can provide opportunities to improve our quality of life.

WHAT IS MODERN DRUG DISCOVERY

In the fields of medicine, biotechnology and pharmacology, drug discovery is the process by which new candidate medications are discovered. Historically, drugs were discovered through identifying the active ingredient from traditional remedies or by serendipitous discovery. Later chemical libraries of synthetic small molecules, natural products or extracts were screened in intact cells or whole organisms to identify substances that have a desirable therapeutic effect in a process known as classical pharmacology. Since sequencing of the human genome which allowed rapid cloning and synthesis of large quantities of purified proteins, it has become common practice to use high throughput screening of large compounds libraries against isolated biological targets which are hypothesized to be disease modifying in a process known as reverse pharmacology. Hits from these screens are then tested in cells and then in animals for efficacy. Even more recently, scientists have been able to understand the shape of biological molecules at the atomic level, and to use that knowledge to design drug candidates (Figure 1).

Modern drug discovery involves the identification of screening hits, medicinal chemistry and optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life), and oral bioavailability. Once a compound that fulfills all of these requirements has been identified, it will begin the process of drug development prior to clinical trials. One or more of these steps may, but not necessarily, involve computer-aided drug design.

Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy, "expensive, difficult, and inefficient process" with low rate of new therapeutic discovery [31]. Out of 10,000 compounds actually synthesised in medicinal chemistry laboratory only 250 compounds can reach preclinical trials. From preclinical trials, only 5 compounds can go for actual clinical trials and finally 1 compound may be approved by FDA. So in short 1 compound out of 10,000 compounds synthesised may get into market i.e. 0.01% of the total compounds get approved. Currently, the research and development cost of each new molecular entity (NME) is approximately US$1.8 billion [32].

Drug targets

The definition of “target” itself is something argued within the pharmaceutical industry. Generally, the "target" is the naturally existing cellular or molecular structure involved in the pathology of interest that the drug-in-development is meant to act on. However, the distinction between a “new” and “established” target can be made without a full understanding of just what a “target” is. This distinction is typically made by pharmaceutical companies engaged in discovery and development of therapeutics. In an estimate, 435 human genome products were identified as therapeutic drug targets of FDA-approved drugs [33].

“Established targets” are those for which there is a good scientific understanding, supported by a lengthy publication history, of both how the target functions in normal physiology and how it is involved in human pathology. This does not imply that the mechanism of action of drugs that are thought to act through a particular established targets is fully understood. Rather, “established” relates directly to the amount of background information available on a target, in particular functional information.

In general, “new targets” are all those targets that are not “established targets” but which have been or are the subject of drug discovery campaigns. These typically include newly discovered proteins, or proteins whose function has now become clear as a result of basic scientific research. The majority of targets currently selected for drug discovery efforts are proteins. Two classes predominate: G-protein-coupled receptors (or GPCRs) and protein kinases.

Screening and design

The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target. For example, if the target is a novel GPCR, compounds will be screened for their ability to inhibit or stimulate that receptor, if the target is a protein kinase, the chemicals will be tested for their ability to inhibit that kinase. It is very unlikely that a perfect drug candidate will emerge from these early screening runs. It is more often observed that several compounds are found to have some degree of activity, and if these compounds share common chemical structural features, one or more pharmacophores can then be developed. At this point, medicinal chemists will attempt to use structure-activity relationships (SAR) to improve certain features of the lead compound:

- Increase activity against the chosen target
- Reduce activity against unrelated targets
- Improve the druglikeness or ADME properties of the molecule.

This process needs several iterative screening runs to improve the properties of the new molecular entities and allow the favoured compounds to go forward to in vitro and in vivo testing for activity in the disease model of choice. Amongst the physico-chemical properties associated with
drug absorption include ionization (pKa), and solubility; permeability can be determined by PAMPA and Caco-2. PAMPA is attractive as an early screen due to the low consumption of drug and the low cost compared to tests such as Caco-2, gastrointestinal tract (GIT) and Blood–brain barrier (BBB) with which there is a high correlation.

A range of parameters can be used to assess the quality of a compound, or a series of compounds, as proposed in the Lipinski’s Rule of Five [34, 35]. Such parameters include calculated properties such as cLogP to estimate lipophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement of enzymatic clearance etc. Some descriptors such as ligand efficiency [36] (LE) and lipophilic efficiency [37,38] (LiPE) combine such parameters to assess druglikeness.

Lipinski’s rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient[39] log P not greater than 5
- Polar surface area equal to or less than 140 Å²

Note that all numbers are multiples of five, which is the origin of the rule’s name [RO5]. In an attempt to improve the predictions of druglikeness, the Lipinski’s rule has spawned many extensions [40]. Also the 500 molecular weight cutoff has been questioned. Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally active and those that are not for a large data set of compounds in the rat [41]. In particular, compounds which meet only the two criteria of:

- 10 or fewer rotatable bonds and
- polar surface area equal to or less than 140 Å² are predicted to have good oral bioavailability [41]

During drug discovery, lipophilicity and molecular weight are often increased in order to improve the affinity and selectivity of the drug candidate. Hence it is often difficult to maintain drug-likeness (i.e., RO5 compliance) during hit and lead optimization. Hence it has been proposed that members of screening libraries from which hits are discovered should be biased toward lower molecular weight and lipophilicity so that medicinal chemists will have an easier time in delivering optimized drug development candidates that are also drug-like. Hence the rule of five has been extended to the rule of three (RO3) for defining lead-like compounds [42].

A rule of three compliant compound is defined as one that has:

- octanol-water partition coefficient log P not greater than 3
- molecular mass less than 300 daltons
- not more than 3 hydrogen bond donors
- not more than 3 hydrogen bond acceptors
- not more than 3 rotatable bonds

While HTS is a commonly used method for novel drug discovery, it is not the only method. It is often possible to start from a molecule which already has some of the desired properties. Such a molecule might be extracted from a natural product or even be a drug on the market which could be improved upon (so-called “me too” drugs). Other methods, such as virtual high throughput screening, where screening is done using computer-generated models and attempting to “dock” virtual libraries to a target, are also often used.

Another important method for drug discovery is drug design, whereby the biological and physical properties of the target are studied, and a prediction is made of the sorts of chemicals that might fit into an active site. One example is fragment-based lead discovery (FBLD). Novel pharmacophores can emerge very rapidly from these exercises. In general, computer-aided drug design is often but not always used to try to improve the potency and properties of new drug leads.

Once a lead compound series has been established with sufficient target potency and selectivity and favourable drug-like properties, one or two compounds will then be proposed for drug development. The best of these is generally called the lead compound, while the other will be designated as the “backup”.

Historical background

The effect of drug in human body are mediated by specific interactions of the drug molecule with biological macromolecules, (proteins or nucleic acids in most cases). This led scientists to the conclusion that individual chemicals are required for the biological activity of the drug. This made for the beginning of the modern era in pharmacology, as pure chemicals, instead of crude extracts, became the standard drugs. Examples of drug compounds isolated from crude preparations are morphine, the active agent in opium, and digoxin, a heart stimulant originating from Digitalis lanata. Organic chemistry also led to the synthesis of many of the cochemicals isolated from biological sources.

Nature as source of drugs

Despite the rise of combinatorial chemistry as an integral part of lead discovery process, natural products still play a major role as starting material for drug discovery [43]. A report was published in 2007, [44] covering years 1981-2006 details the contribution of biologically occurring chemicals in drug development. According to this report, of
the 974 small molecule new chemical entities, 63% were natural derived or semisynthetic derivatives of natural products. For certain therapy areas, such as antimicrobials, antineoplastics, antihypertensive and anti-inflammatory drugs, the numbers were higher. In many cases, these products have been used traditionally for many years. Natural products may be useful as a source of novel chemical structures for modern techniques of development of antibacterial therapies [45]. Despite the implied potential, only a fraction of Earth’s living species has been tested for bioactivity.

Plant-derived medicines

Prior to Paracelsus, the vast majority of traditionally used crude drugs in Western medicine were plant-derived extracts. This has resulted in a pool of information about the potential of plant species as an important source of starting material for drug discovery. A different set of metabolites is sometimes produced in the different anatomical parts of the plant (e.g. root, leaves and flower), and botanical knowledge is crucial also for the correct identification of bioactive plant materials.

Microbial metabolites as source of antibiotics

Microbes compete for living space and nutrients. To survive in these conditions, many microbes have developed abilities to prevent competing species from proliferating. Microbes are the main source of antimicrobial drugs. *Streptomyces* species have been a valuable source of antibiotics. The classical example of an antibiotic discovered as a defense mechanism against another microbe is the discovery of penicillin in bacterial cultures contaminated by *Penicillium* fungi in 1928.

Marine invertebrates as source of new medicines

Marine environments are potential sources for new bioactive agents [46]. Arabinose nucleosides discovered from marine invertebrates in 1950s, demonstrating for the first time that sugar moieties other than ribose and deoxyribose can yield bioactive nucleoside structures. Ziconotide was the first marine-derived drug approved in 2004 (figure 2).

The cone snail toxin ziconotide, also known as Prialt, was approved by the Food and Drug Administration to treat severe neuropathic pain. Several other marine-derived agents are now in clinical trials for indications such as cancer, anti-inflammatory use and pain. One class of these agents are bryostatin-like compounds, under investigation as anti-cancer therapy.

Chemical diversity of natural products

Combinatorial chemistry was a key technology enabling the efficient generation of large screening libraries for the needs of high-throughput screening. However, now, after two decades of combinatorial chemistry, it has been pointed out that despite the increased efficiency in chemical synthesis, no increase in lead or drug candidates have been reached [44].

This has led to an analysis of chemical characteristics of combinatorial chemistry products, compared to existing drugs or natural products. The chemoinformatics concept of chemical diversity, depicted as distribution of compounds in the chemical space based on their physicochemical characteristics, is often used to describe the difference between the combinatorial chemistry libraries and natural products.

The synthetic, combinatorial library compounds seem to cover only a limited and quite uniform chemical space, whereas existing drugs and particularly natural products, exhibit much greater chemical diversity, distributing more evenly to the chemical space [43]. The most prominent differences between natural products and compounds in combinatorial chemistry libraries is the number of chiral centers (much higher in natural compounds), structure rigidity (higher in natural compounds) and number of aromatic moieties (higher in combinatorial chemistry libraries). Other chemical differences between these two groups include the nature of heteroatoms (O and N enriched in natural products, and S and halogen atoms more often present in synthetic compounds), as well as level of non-aromatic unsaturation (higher in natural products). As both structure rigidity and chirality are both well-established factors in medicinal chemistry known to enhance compounds specificity and efficacy as a drug, it has been suggested that natural products compare favourable to today's combinatorial chemistry libraries as potential lead molecules.

Natural product drug discovery

A natural product is a chemical compound or substance produced by a living organism – found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by multistep organic total synthesis.

These small molecules provide the source or substance for the majority of FDA-approved agents and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life-threatening conditions. Natural products may be extracted from tissues of terrestrial plants, marine organisms or microorganisms fermentation broths. A crude (untreated) extract from any one of these sources typically contains novel, structurally diverse chemical compounds, which the natural environment is a rich source of.

Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as bioprospecting.
Screening of natural products for identification of new drug molecules

Pharmacognosy provides the tools to identify select and process natural products destined for medicinal use. Usually, the natural product compound has some form of biological activity and that compound is known as the active principle - such a structure can act as a “lead compound”. Many of today's medicines are obtained directly from a natural source.

On the other hand, some medicines are developed from a “lead compound” originally obtained from a natural source. This means the lead compound:
- can be produced by total synthesis, or
- can be a starting point (precursor) for a semisynthetic compound, or
- can act as a template for a structurally different total synthetic compound.

This is because most biologically active natural product compounds are secondary metabolites with very complex structures. This has an advantage in that they are novel compounds but this complexity also makes many lead compounds' synthesis difficult and the compound usually has to be extracted from its natural source – a slow, expensive and inefficient process. As a result, there is usually an advantage in designing simpler analogues.

The plant kingdom as source of natural products

Plants have always been a rich source of lead compounds (e.g. Alkaloids, morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine). Many of these lead compounds are useful drugs in themselves (e.g. Alkaloids, morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anaesthetics developed from cocaine). Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the antimalarial agent artemisinin from Artemisia annua.

Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in laboratories. Furthermore, evolution has already carried out a screening process itself whereby plants are more likely to survive if they contain potent compounds which deter animals from eating them. Even today, the number of plants that have been extensively studied is relatively very few and the vast majorities have not been studied at all. Major classes of molecules include terpenoids, phytosterols, alkaloids, natural phenols and polyphenols.

The microbial world as source of natural products

Microorganisms such as bacteria and fungi have been invaluable for discovering drugs and lead compounds. These microorganisms produce a large variety of antimicrobial agents which have evolved to give their hosts an advantage over their competitors in the microbiological world.

The screening of microorganisms became highly popular after the discovery of penicillin. Soil and water samples were collected from all over the world in order to study new bacterial or fungal strains, leading to an impressive arsenal of antibacterial agents such as the cephalosporins, tetracyclines, aminoglycosides, rifamycins, and chloramphenicol.

The marine world as source of natural products

In recent years, there has been a great interest in finding lead compounds from marine sources. Coral, sponges, fish, and marine microorganisms have a wealth of biologically potent chemicals with interesting inflammatory, antiviral, and anticancer activity. For example, curacin A is obtained from a marine cyanobacterium and shows potent antitumor activity. Other antitumor agents derived from marine sources include eleutherobin, discodermolide, bryostatins, dolostatins, and cephalostatins.
Animal sources for natural products

Animals can sometimes be a source of new lead compounds. For example, a series of antibiotic peptides were extracted from the skin of the African clawed frog and a potent analgesic compound called epibatidine was obtained from the skin extracts of the Ecuadorian poison frog. It is 200 times as potent as morphine. Secretions from dendrobatids are also showing promise as muscle relaxants and heart stimulants. [48, 49] This Phantasmal poison frog lives up to ten years in captivity. This species is endangered, and there are only seven known locations in the wild to find this extraordinary frog.

Epibatidine an alkaloid obtained from Ecuadorian poison frog

Morphine is a potent opiate analgesic drug obtained from Opium poppy fruits

Exenatide is a 39-amino acid peptide derived from saliva of Gila Monster

Exenatide, derived from a compound found in the saliva of the Gila monster, a large lizard native to the southwestern US, is a functional analog of Glucagon-Like Peptide-1 (GLP-1), a naturally occurring peptide [50]. Exenatide is a 39-amino-acid peptide, an insulin secretagogue, with glucoregulatory effects. It was approved in April 2005 for the treatment of diabetes mellitus type 2. It belongs to the group of incretin mimetics and is manufactured by Amylin Pharmaceuticals. While it has blood-sugar lowering actions alone, it can also be combined with other medications such as pioglitazone, metformin, sulfonylureas, and/or insulin to improve glucose control.

Exenatide is a synthetic version of exendin-4, a hormone found in the saliva of the Gila monster that was first isolated by Dr. John Eng in 1992 while working at the Veterans Administration Medical Center in the Bronx, New York. It displays biological properties similar to human glucagon-like peptide-1 (GLP-1), a regulator of glucose metabolism and insulin secretion. According to the package insert, exenatide enhances glucose-dependent insulin secretion by the pancreatic beta-cell, suppresses inappropriately elevated glucagon secretion, and slows gastric emptying, although the mechanism of action is still under study. A once-weekly injection has been approved as of January 27, 2012 under the trademark Bydureon [51].

Venoms and toxins as source of natural products

Venoms and toxins from animals, plants, snakes, spiders, scorpions, insects [33] and microorganisms are extremely potent because they often have very specific interactions with a macromolecular target in the body. As a
result, they have proved important tools in studying receptors, ion channels, and enzymes. Many of these toxins are polypeptides (e.g. α-bungarotoxin from cobras). However, non-peptide toxins such as tetrodotoxin from the puffer fish are also extremely potent.

Venoms and toxins have been used as lead compounds in the development of novel drugs. For example, teprotide, a peptide isolated from the venom of the Brazilian viper, was the lead compound for the development of the antihypertensive agent’s cilazapril and captopril. The neurotoxins from *Clostridium botulinum* are responsible for serious food poisoning (botulism), but they have a clinical use as well. They can be injected into specific muscles (such as those controlling the eyelid) to prevent muscle spasm. These toxins prevent cholinergic transmission and could well prove a lead for the development of novel anticholinergic drugs.

**Isolation and purification of natural products**

If the lead compound (or active principle) is present in a mixture of other compounds from a natural source, it has to be isolated and purified. The ease with which the active principle can be isolated and purified depends much on the structure, stability, and quantity of the compound. For example, Alexander Fleming recognized the antibiotic qualities of penicillin and its remarkable non-toxic nature to humans, but he disregarded it as a clinically useful drug because he was unable to purify it. He could isolate it in aqueous solution, but whenever he tried to remove the water, the drug was destroyed. It was not until the development of new experimental procedures such as freeze drying and chromatography that the successful isolation and purification of penicillin and other natural products became feasible.

**Medicinal Chemistry - Multistep Organic Synthesis, Synthetic Organic Chemistry/ NCE Research- Medicinal Chemistry [51a]**

Elaborate

Not all natural products can be fully synthesized and many natural products have very complex structures that are too difficult and expensive to synthesize on an industrial scale. These include drugs such as penicillin, morphine, and paclitaxel (Taxol) [7]. Many higher plants contain novel metabolites with antimicrobial and antiviral properties. However, in the developed world almost all clinically used chemotherapeutics have been produced by *in vitro* chemical synthesis. Exceptions, like taxol and vincristine, were structurally complex metabolites that were difficult to synthesize *in vitro*. Many non-natural, synthetic drugs cause severe side effects that were not acceptable except as treatments of last resort for terminal diseases such as cancer. The metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs, because they must accumulate within living cells [43].

Taxol, which was originally isolated from the bark of the Pacific yew tree, is now the product of a four-step semisynthesis that starts with a compound derived from the needles of the more common English yew tree. Paclitaxel is manufactured by extracting 10-deacetylbaccatin III from the needles of the yew tree, then carrying out a four-stage synthesis as shown in following figure. The semisynthesis was developed and patented by Florida State University [52].

**Drugs derived from Natural lead molecules**

**Screening (Table 3)**

Two main approaches exist for the finding of new bioactive chemical entities from natural sources. The first is referred to as random collection and screening of material. This approach is based on the fact that only a small part of earth’s biodiversity has ever been tested for pharmaceutical activity and organisms living in a species-rich environment need to evolve defensive and competitive mechanisms to survive. A collection of plant, animal and microbial samples from rich ecosystems can potentially give rise to novel biological activities worth exploiting in the drug development process. One example of a successful use of this strategy is the screening for antitumour agents by the National Cancer Institute, USA to jointly develop the Taxol molecule for commercial purpose for the benefit of society.
Paclitaxel showed anti-tumour activity by a previously unknown mechanism i.e. stabilization of microtubules. It is now approved for clinical use for the treatment of lung, breast and ovarian cancer, as well as for Kaposi’s sarcoma.

Cabazitaxel is a semi-synthetic derivative of the natural taxoid 10-deacetylbaccatin III with potential antineoplastic activity. Cabazitaxel binds to and stabilizes tubulin, resulting in the inhibition of microtubule depolymerization and cell division, cell cycle arrest in the G2/M phase, and the inhibition of tumor cell proliferation. Cabazitaxel has been shown effective against prostate cancer, also because it works by preventing the formation of microtubules, which pull the chromosomes apart in dividing cells (such as cancer cells). Still another examples are:

1. Camptotheca (Camptothecin, Topotecan, Irinotecan, Rubitecan, Belotecan);
2. Podophyllum (Etoposide, Teniposide);
3a. Anthracyclines (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, Zorubicin);
3b. Anthracenediones (Mitoxantrone, Pixantrone).

In China, Camptotheca tree was in use as traditional folk medicine for cure of cancer. An active principle Camptothecin, a quinoline alkaloid was isolated from the bark and stem of this tree. In modern times several highly potent anticancer medicines like Topotecan, Irinotecan, Belotecan were developed based on the core structure of Camptotherin. Therefore, camptothecin is a lead compound in this case, which gave further direction to development of its synthetic analogues. These compounds acts as inhibitor of topoisomerase which prevents DNA formation in cancerous cell thus stopping their proliferation and controls stops the growth of cancerous cells.

Vinblastine and vincristine are alkaloids found in the Madagascar periwinkle, Catharanthus roseus (formerly known as Vinca rosea)[64].

The natives of Madagascar traditionally used the vinca rosea to treat diabetes. In fact it has been used for centuries throughout the world to treat all kinds of ailments
from wasp stings, in India, to eye infections in the Caribbean.

When researchers began to analyse the plant in the 1950’s they discovered it contained over 70 alkaloids. Some were found to lower blood sugar levels and others to act as hemostatics, but the most interesting were vinblastine and vincristine, which were found to lower the number of white cells in blood. A high number of white cells in the blood indicates leukemia. So a new anti-cancer drug had been discovered[65,66].

They work by preventing mitosis in metaphase. These alkaloids bind to tubulin, thus preventing the cell from making the spindles it needs to be able to divide. This is different from the action of taxol which interferes with cell division by keeping the spindles from being broken down[67].

Vinblastine is mainly useful for treating Hodgkin’s disease, advanced testicular cancer and advanced breast cancer. Vincristine is mainly used to treat acute leukemia and other lymphomas[68].

Vinblastine was first isolated by Robert Noble and Charles Thomas Beer at the University of Western Ontario from the Madagascar periwinkle plant. Vinblastine’s utility as a chemotherapeutic agent was first suggested by its effect on the body when the plant was consumed in a tea. Drinking the tea led to a decreased number of white blood cells, so it was hypothesized that vinblastine might be effective against cancers of the white blood cells such as lymphoma.

Vinblastine may be isolated from the Madagascar Periwinkle (Catharanthus roseus), along with several of its precursors- catharanthine and vindoline. Extraction is costly and yields of vinblastine and its precursors are low. Enantioselective synthesis has been of considerable interest in recent years, as the natural mixture of isomers is not an economical source for the required C16’S, C14’R stereochemistry of biologically active vinblastine. Initially, the approach depends upon an enantioselective Sharpless epoxidation, which sets the stereochemistry at C20. The desired configuration around C16 and C14 can then be fixed during the ensuing steps. In this pathway, vinblastine is constructed by a series of cyclization and coupling reactions which create the required stereochemistry. The overall yield may be as great as 22%, which makes this synthetic approach more attractive than extraction from natural sources, whose overall yield is about 10%[69]. Stereochemistry is controlled through a mixture of chiral agents (Sharpless catalysts), and reaction conditions (temperature, and selected enantiopure starting materials)[70].

**Present Approach/ Our approach.**

Among the various natural products, Coumarins moieties are reported to have shown excellent biological activities. Umbelliferone, also known as 7-hydroxycoumarin, hydrangine, skimmetine, and beta-umbelliferone, is a widespread natural product of the coumarin family [71].

It absorbs ultraviolet light strongly at several wavelengths. Despite several indications that this chemical is photomutagenic, it is used in sunscreens[71]. Umbelliferone has been reported to have antioxidant properties[72].

**Umbelliferone occurs in many familiar plants from the Apiaceae (Umbelliferae) family such as carrot, coriander and garden angelica, as well as in plants from other families, such as the mouse-ear hawkweed (Hieracium pilosella, Asteraceae) or the bigleaf hydrangea (Hydrangea macrophylla, Hydrangeaceae, under the name hydrangine). It is one of the components of asafoetida, the dried latex from the giant fennel (Ferula communis). Umbelliferone is the parent compound for a large number of natural products. Herniarin or 7-O-methylumbelliferone (7-methoxycoumarin) occurs in the leaves of water hemp (Eupatorium ayapana) and rupturewort. O-glycosylated derivatives such as skimmin (7-O-β-D-glucopyranosylumbelliferone) occur naturally and are used for the fluorimetric determination of glycoside hydrolase enzymes. Isoprenylated derivatives are also widespread, such as marmin (found in grapefruit skin and in the bark of the Bael tree) and furocoumarins such as marmesin, angelicin, and psoralen.**

**Umbelliferone 7-apiosylglucoside can be isolated from the root of Gmelina arborea [72].**

In our literature search with respect to 4-Methyl umbelliferone, we found that this structural scaffold has
exhibited anti cancer activity [71]. 4-Methyl umbelliferone, a modified coumarin (7-Hydroxy-4-methyl coumarin) has been used as folk oral medicine in Japan [72]. Hymecromone (4-methylumbelliferone) is a drug used in bile therapy. It is used as choleretic and antispasmodic drugs and as a standard for the fluorometric determination of enzyme activity. A more soluble form of hymecromone is disclosed in EP-A-0240874 and tablets of hymecromone for improving the excretion of bile are known from U.S. Pat. No.3,175,943. The choleretic and biliary antispasmodic activity of hymecromone also is referred to in Petrioli. Hymecromone is also available as a natural occurring extract of Manna Ash known as Fraxin.

This directed our attention towards making some transition metal conjugates and explore the biological activity / anticancer activity using schiffs base chemistry. Metallorganic chemistry is widely being explored by research scientists in drug discovery and development programmes worldwide. The ease of synthesis and availability of raw materials like heterocyclic aldehydes and heterocyclic aniline, aliphatic diamino compounds leads to various possibilities of generating novel metal conjugates by permutation and combination. With this approach in mind, we decided to synthesis substituted ortho hydroxyl benzaldehydes and suitably substituted anilines and diamino compounds.

For the synthesis of novel schiffs base, we need to have either a novel aldehyde or novel amino/diamino compound. It may not be possible to have both novel compounds due to tremendous research work has already been reported in literature. Our initial attempts to synthesis completely novel aniline compounds, starting from substituted nitro benzenes to get substituted nitrocoumarins, were not successful due to electron withdrawing nitro group on benzene ring, hence cyclisation reactions/ Pechmann condensation under various conditions were not successful and we ended with recovery of starting material. Hence synthesizing a completely novel amino/diamino compound and completely novel orthohydroxy benzaldehydes was a limitation in our research laboratory. Hence, we thought of synthesizing compounds, which require cheap starting materials and simple synthesis procedures and well established and yielding highly pure compounds.

Our second attempt to synthesis, a novel amino compound starting with 4-Hydroxy coumarin was also not yielding desired product. We attempted to condense 4-Hydroxy coumarin with 2-chloroethylamine hydrochloride using various bases like potassium carbonate, sodium hydride in solvents like anhydrous acetone and anhydrous dimethyl formamide were not successful. Hence this route was also abandoned.

In our third attempt, we could successfully synthesis an aldehyde on coumarin moiety and condense with N-methyl propylene diamine to get a novel schiffs base. This schiffs base was a liquid compound and could not be characterized by spectroscopic techniques like $^1$H NMR and MS. Hence, we converted this oily compound into its oxalate salt by employing its tertiary nitrogen atom having dimethyl group available for protonation with oxalic acid. This yielded us a solid compound which could be dried well and non-hygrosocopic yellow powder, stable at room temperature and humidity conditions was isolated. This was characterized well using $^1$H NMR, $^{13}$C NMR MS and IR spectroscopy.

Later on, schiffs base of 4-methyl-7-hydroxy-8-formyl coumarin with N-methyl propylene diamine was generated in situ and condensed with metal chlorides in ethanol at reflux temperature to get its metal conjugates. Metal complexes with ZnCl$_2$, CuCl$_2$, NiCl$_2$ and CoCl$_2$ were synthesized.

Using Similar strategy another series of schiffs base metal conjugates using 4-methyl-7-hydroxy-8-formyl coumarin with N, N-dimethyl propylene diamine was generated. The schiffs base was also converted into its oxalate salt for spectroscopic characterization by $^1$H NMR, $^{13}$C NMR MS and IR spectroscopy[74].

The biological activity of these complexes was also studied in vitro against organism Escherichia coli for antibacterial activity and Escherichia coli for antifungal activity and antimycobacterium activity against mycobacterium tuberculosis[75].

Our next strategy was to synthesis an aldehyde Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methyl-1,3-thiazole-5-carboxylate as per [procedure reported in literature and condense with substituted heterocyclic anilines like 4-(1H-1,2,4-triazol-1-yl methyl) aniline, 4-(4-aminobenzyl)-1,3-oxazolidine-2-one and 2-Butyl-5-amino-1-benzofuran. Ethyl 2’(3<formyl4 hydroxyphenyl)-4 methyl 1,3 thiabole 5’ carboxylate was also condensed with N, N-dimethyl propylene diamine to form corresponding Schiff base. Thus four novel Schiff bases were synthesized and characterized by spectroscopic techniques. These novel Schiff bases were further complexed with metal chlorides such as ZnCl$_2$, CuCl$_2$, NiCl$_2$. [Scheme 2].

The antituberculosis activity of these complexes was also studied in vitro against mycobacterium tuberculosis. Also substituted heterocyclic anilines like 4-(1H-1,2,4-triazol-1-yl methyl) aniline, 4-(4-aminobenzyl)-1,3-oxazolidine-2-one and 2-Butyl-5-amino-1-benzofuran were condensed with salicylaldehyde to get novel Schiff bases and their transition metal complexes. The antituberclosis activity of these complexes was also studied in vitro against mycobacterium tuberculosis [ Scheme 3].

Other permutation and combination of aldehyde compounds with available heterocyclic anilines were also done to synthesis novel schiffs bases and their corresponding transition metal complexes were also prepared and biological activities were studied. These compounds were characterized well using $^1$H NMR, $^{13}$C NMR, MS and IR spectroscopy.

RESULTS AND DISCUSSION
**In-vitro antimicrobial activity**

We found that comparative study of MIC values of Schiff base and its complexes indicated metal complexes exhibit higher antimicrobial activity than the free Schiff base ligands and the same is indicated from the results given in the Table 4.

There was no promising antibacterial activity observed against gram negative bacteria i.e. *E. Coli* and *Pseudomonas*. It was in the range of MIC value 50-100 µg/ml concentration compared to standard antibiotic Ciprofloxacin having MIC of 2µg/mL. This may be due to effective barrier of an outer membrane of gram negative bacteria, towards intake of external substances like test compounds under this study.

The sensitivity of the test organisms to the test compounds may also be associated with cell wall structure. The major role of action involves highly specific coordination of metal ion to thiol groups on proteins containing L-cysteine[76]. The reduced activity of the test compounds may be due to lack of such coordination of Zn(II) to form a specific complex with cell wall protein thiol groups. However, in case of *S. aureus*, [Zn(NMAPIMHMC)₂].2H₂O complex showed moderate activity upto MIC value of 12.5µg/mL and [Zn(TMPIMP)₂].2H₂O complex showed activity upto MIC value of 6.25µg/mL. This could be due to coordination of Zn(II) atom to form a specific complex with cell wall protein thiol groups and ultimately interfering in cell wall synthesis of *S. aureus* during cell multiplication phase. The observed activity of the test compounds indicates the future potential for the development of metal coordination complexes to overcome the limitations due to currently available antibiotics to treat MSRA. In case of antifungal activity against *C. albicans*, [Zn(NMAPIMHMC)₂].2H₂O and [Zn(TMPIMP)₂].2H₂O complex showed most promising activity upto MIC value of 3.12µg/mL, compared to standard Fluconazole having MIC value 16µg/mL.

However, in case of antifungal activity against *A. niger*, Zn(NMAPIMHMC)₂].2H₂O showed the most promising activity upto MIC value of 0.8 µg/mL compared with standard Fluconazole having MIC value 8µg/mL. [Zn(TMPIMP)₂].2H₂O and [Zn(HBABO)₂].2H₂O complexes also showed better activity upto MIC value of 3.12 µg/mL.

In almost all the comparative studies done, metal complexes showed enhanced activity compared with Schiff base ligand. These observations are due to heterocyclic rings of coumarin moeity, triazole heterocyclic ring and oxathiazolidinone heterocyclic ring incorporated in the molecular structure of the metal complexes. These structural scaffolds might interfere in the mechanism of cell multiplication as discussed above and hence stop further growth of fungus.

**In vitro anti-tuberculosis activity**

In order to begin our efforts for such new medicines as effective anti tuberculosis agents against *M. Tuberculosis*, we thought of combining heterocyclic aniline scaffold with simple ortho hydroxy benzaldehydes like salicylaldehyde to get a Schiff base and its conversion to Zn (II) metal complex. Recent literature survey for transition metal complexes as anti tuberculosis agents in this field gave few references [77-80] indicating possibility of getting new lead molecules in this field. Novel anti-TB drugs, which are safe, able to shorten the course of treatment, effective against drug-resistant strains and latent TB infection, are urgently needed, especially in the era of MDR- and XDR-TB. The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA)[81].

**Table 1. Generic Name**

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Year</th>
<th>Indication</th>
<th>Innovator company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orlistat</td>
<td>1999</td>
<td>obesity</td>
<td>Roche</td>
</tr>
<tr>
<td>Miglitol</td>
<td>1996</td>
<td>antidiabetic (Type II)</td>
<td>Bayer</td>
</tr>
<tr>
<td>Topotecan</td>
<td>1996</td>
<td>antineoplastic</td>
<td>SmithKline Beecham</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>1995</td>
<td>antineoplastic</td>
<td>Rhône-Poulenc Rorer</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1993</td>
<td>immunosuppressant</td>
<td>Fujisawa</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1993</td>
<td>antineoplastic</td>
<td>Bristol-Myers Squibb</td>
</tr>
</tbody>
</table>

**Table 2. Natural products currently being evaluated as potential drugs[60]**

<table>
<thead>
<tr>
<th>Natural product</th>
<th>Source</th>
<th>Target</th>
<th>Indication</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>manolalide</td>
<td>marine sponge</td>
<td>phospholipage-A2 Ca2+-release</td>
<td>anti-inflammatory</td>
<td>clinical trials</td>
</tr>
<tr>
<td>dolastatin</td>
<td>sea hare</td>
<td>microtubules</td>
<td>antineoplastic,</td>
<td>nonclinical</td>
</tr>
<tr>
<td>staurosporine</td>
<td>streptomyces</td>
<td>protein kinase C</td>
<td>antineoplastic</td>
<td>clinical trials</td>
</tr>
<tr>
<td>epothilone</td>
<td>mycobacterium</td>
<td>microtubules</td>
<td>antineoplastic</td>
<td>research</td>
</tr>
<tr>
<td>calanolide A, B</td>
<td>tree</td>
<td>DNA polymerase action on reverse transcriptase</td>
<td>acquired immunodeficiency syndrome (AIDS)</td>
<td>clinical trials</td>
</tr>
<tr>
<td>huperzine A</td>
<td>moss</td>
<td>cholinesterase</td>
<td>alzheimer’s disease</td>
<td>clinical trials</td>
</tr>
</tbody>
</table>
Table 3. Following drugs have been derived from biological sources in nature [31]

<table>
<thead>
<tr>
<th>Drug</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>Ergotamine [56]</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Ertapenem</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Etoposide</td>
</tr>
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<td>Cetuximab</td>
<td>Etoposide</td>
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<td>Cetuximab</td>
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<td>Etoposide</td>
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<tr>
<td>Cetuximab</td>
<td>Etoposide</td>
</tr>
</tbody>
</table>

Table 4. showing comparative antibacterial and antifungal screening results by MIC method [75]

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Test organism and sample concentration in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>NMAPIMHMC.oxalate</td>
<td>50</td>
</tr>
<tr>
<td>[Zn(NMAPIMHMC)2]•2H2O</td>
<td>50</td>
</tr>
<tr>
<td>TMPIMP</td>
<td>100</td>
</tr>
<tr>
<td>HBABO</td>
<td>100</td>
</tr>
<tr>
<td>[Zn(HBABO)2]•2H2O</td>
<td>100</td>
</tr>
<tr>
<td>Std. Ciprofloxacin</td>
<td>2</td>
</tr>
<tr>
<td>Std. Fluconazole</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Anti-TB activity screening results by MIC method

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Test sample</th>
<th>MIC in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.Tuberculosis</td>
<td>Zn(DMAPIMMMC)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>[Zn(NMAPIMHMC)2]•2H2O</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>[Zn(HBABO)2]•2H2O</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>[Zn(BBFIMP)2]•2H2O</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Standard Pyrazinamide</td>
<td>3.125</td>
</tr>
<tr>
<td></td>
<td>Standard Streptomycin</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Fig 1. Overall flow chart of modern drug discovery

Fig 2. Ziconotide (SNX-111; Prialt) is a non-opioid and non-NSAID analgesic agent used for the amelioration of severe and chronic pain. Derived from Conus magus (“Cone Snail”), it is the synthetic form of an ω-conotoxin peptide [47]

Fig 3. Paclitaxel is a mitotic inhibitor used in cancer chemotherapy. It was discovered in a US National Cancer Institute program at the Research Triangle Institute in 1967 when Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific yew tree, Taxus brevifolia and named it taxol. Later it was discovered that endophytic fungi in the bark synthesize paclitaxel.

Fig 4. Cabazitaxel (previously XRP-6258, trade name Jevtana) is a semi-synthetic derivative of a natural taxoid [62]. It was developed by Sanofi-Aventis and was approved by the U.S. Food and Drug Administration (FDA) for the treatment of hormone-refractory prostate cancer on June 17, 2010. It is a microtubule inhibitor, and the fourth taxane to be approved as a cancer therapy [63]. Cabazitaxel in combination with prednisone is a treatment option for hormone-refractory prostate cancer following docetaxel-based treatment.
Fig 5. Comparative Anti-TB activity results by MIC method

Scheme 1. Synthesis of Schiff base having coumarin core and their transition metal complexes [73]

Scheme 2. Synthesis of Schiff base having thiazole core and their transition metal complexes

Scheme 3. Synthesis of novel heterocyclic schiffs bases and their transition metal complexes
A comparison of the metal complexes with that of reference Pyrazinamide and Streptomycin showed that the antituberculosis activity of the metal complexes was moderate. This could be due to heterocyclic rings present in the molecular structure of the metal complexes. The results of the studies of minimum inhibitory concentration of the metal complexes are summarized in Table 5 and graphically represented in Figure 5. These Zinc(II) complexes have shown moderate antimycobacterium activity as compared to standard Pyrazinamide and Streptomycin against M. Tuberculosis. This could be attributed to the fact of low permeability of mycobacterial cell wall towards compounds being studied [82-85]. The action and mechanism of resistance to the antituberculosis drugs are still not understood. However, molecular perceptive of the drug resistance and drug action in M. tuberculosis may eventually lead to rational drug design of new anti-TB drugs [86].

CONCLUSION

With this research finding, we want to conclude that continuous efforts are necessary to explore innovative ways to explore possibilities of discovering new chemical entities by simple and efficient synthesis and isolation procedures. Research students working in colleges of Indian universities and in other institutes having limited resources, may adopt our approaches to pursue their research objectives. With such simple methods and systematic studies, there is a possibility to discover new compounds useful as very effective medicines in certain therapeutic classes as antiretroviral, antimarial, anticancer etc.

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