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Chapter 4

Reverse Pharmacology

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INTRODUCTION AND BACKGROUND

> *I never found it [drug discovery] easy. People say I was lucky twice but I resent that. We stuck with [cimetidine] for 4 years with no progress until we eventually succeeded. It was not luck, it was bloody hard work.*
>
> — Sir James Black, Nobel Laureate (Jack, 2009).

Introduction

The aforementioned quote from Sir James Black, the discoverer of β-adrenergic and H_2- blockers, expresses the exasperation so often felt by many scientists who have dedicated their lives to new drug discoveries. Any new drug discovery for an unmet medical need often grabs headlines in the medical and lay press/media. Overenthusiastic science writers, not infrequently, depict the epitome of medical progress in superlative terms. The current drug discovery and development processes depend heavily on reductionist paradigms. The latter involve identification of drug targets, synthesizing many molecules through combinatorial chemistry/monoclonal antibodies, and testing the drug candidates through high throughput or binding to specific macromolecules. Despite the advances in genomics, proteomics, and metabolomics, the new drug development is costly, time-consuming, less productive, and yields low returns on heavy investments. The current statistics indicate a drying up of the drug discovery pipeline (New Drug Discovery: in 1996 (56), 2007 (17), 2013 (03)) (Mishra, 2014). There is also a further aggravation in the drug supply due to some of the marketed blockbuster drugs that have been withdrawn. There are opinions expressed about the need of a paradigm shift in the processes of drug discovery and development. The transdiscipline of reverse pharmacology (RP)

Innovative Approaches in Drug Discovery. DOI: http://dx.doi.org/10.1016/B978-0-12-801814-9.00004-0

offers one such opportunity to shift the paradigm, with an eye to saving cost and time—and with greater chances of success in clinical therapeutics.

Background of Reverse Pharmacology

The large number of the world population (about 70%) still relies on traditional systems of medicine (TSM). In India, more than 500 million people turn to various forms of medicine such as Ayurveda, Unani, Siddha, and homeopathy (AYUSH) (Raut, 2013). The major components of the remedies in the TSM pharmacopoeias are medicinal plants. There are increasing global demands of safe herbal products for many intercurrent and chronic illnesses. Quite a number of Ayurvedic and traditional Chinese medicine (TCM) plants sell in the Western markets as dietary supplements (DS), as over-the-counter drugs, with vague claims of structure and function improvement. There is a disclaimer on every bottle that the United States Federal Drug Administration (USFDA) has not approved the DS. This dichotomy of not accepting the systems of health care (Ayurveda or TCM) and permitting the drugs of those systems as DS is hazardous! More than 100 countries have some form of regulations for herbal medicines. The market for herbal supplements and medicine is expected to cost approximately US $100 billion by 2015 (Stephen Daniells, 2011). There is a World Health Organization (WHO) projection of the global herbal market to reach the figure of $5 trillion by 2050 (India Medical Times). The evidence base of the herbal remedies is considered inadequate despite their long use. What these remedies need are different kinds of evidence—and a different approach. When these age-old natural drugs are taken out of their contextual matrix and subjected to significant statistical studies, their "Cochrane review" often pronounces them as "not convincing" statistically. It is interesting to note what Sir Bradford Hill, the pioneer of controlled clinical trials, said,

> *When I got on to the subject of statistical tests of significance I started by stating that these were based on the laws of probability over which statisticians quarreled violently. I was entirely ignorant of them but I knew more than the lady who congratulated her friend on the birth of triplets. "It is remarkable," said the mother. "It happens only one in 8000 times." "Good gracious," said her friend. "However did you find time for the housework?*
>
> (Chalmers, 2003)

Reverse pharmacology is essentially a transdisciplinary quest for clinical significance, not merely a matter of flipping a coin. The potential of the widely used TSM drugs/plants can be investigated by RP, which is a novel initiative that offers a paradigm shift in the new drug discovery process. The ingenuity of RP is meant for the integration of traditional remedies with the wisdom of robust documentation of safety and efficacy. RP is staged as experiential knowledge/data, exploratory research, and relevant clinical/experimental studies. Unlike conventional drug discovery and development, RP approach is

done from "bedsides to benches." It can also be relevant for new uses of old drugs or for following up on a new unseen indication of a drug candidate. The clinically novel biodynamic actions of traditional remedies may open up new vistas in biomedicine and life sciences. The phytoactive molecules can also provide novel chemical scaffolds for the structural modifications with defined drug targets. Hence, RP can play a dual role—it can inspire new drugs from traditional remedies and enrich the chemical repertoire of medicinal chemists for new chemical entities (NCEs).

THE ROOTS OF MODERN DRUGS IN TRADITIONAL REMEDIES/POISONS

Around half a century before Jesus, Aulus Cornelius Celsus made a profound statement, "That medicines were first found out, and then after the reasons and causes were discoursed; and not the causes first found out, and by light from them the medicines and cures discovered." Unlike this view, it has been emphasized in Ayurveda that the disease is first investigated and only later the medicines: "*Rogamadau parikshet tadanantaram aushadham*." This obvious difference is primarily due to different epistemologies of the genesis of Greek medicine and that of Ayurveda. As a consequence, the renaissance period of medicine, in Europe, reacted to the Greek physician Galen and his centuries-old entrenched therapeutic concoctions.

Liberated from a blind following, questions were then asked by inquisitive minds about how the tribal poisons worked. This habit of asking how led to developments far beyond the then state of technology. Claude Bernard, the father of physiology and pharmacology, solved the mystery of curare arrow

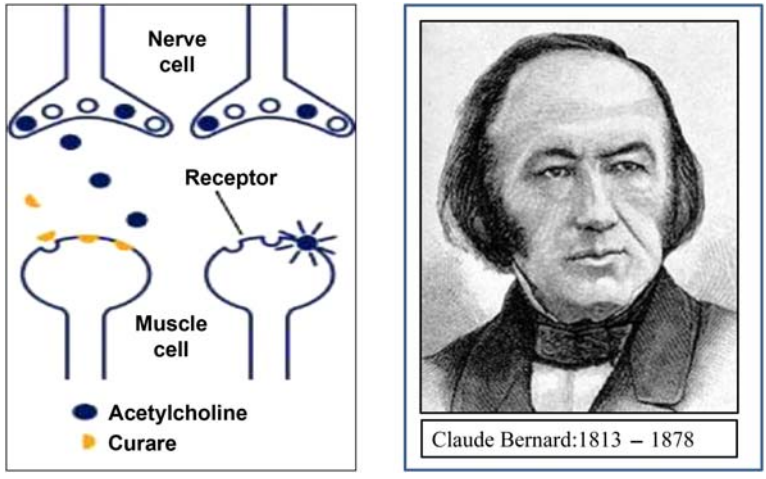


FIGURE 4.1 From arrow poisoning to drug receptor.

TABLE 4.1 Poisons and Mechanisms of Actions (Vaidya, A.D.B., 2010b)

Medicinal Plant	Clinical Effect	Mechanism
Curare tomentosum	Conscious paralysis & death	Neuromuscular block
Papaver somniferum	Sleep & pain relief	Opioid receptors/ endorphins
Physostigma venenosum	Ordeal poison	Anticholineesterase
Claviceps purpurea	Gangrene	α & β adrenergic receptors
Strychnos nux-vomica	Convulsant	Glycinergic receptors
Atropa belladonna	Fatal poisoning	Cholinergic blockade
Melilotus alba	Bleeding disorder	Anti-Vitamin K
Bathraps jaraca	Snake poisoning	ACE inhibition

poison (Fig. 4.1). The victim stays fully conscious but dies of paralysis. Bernard's experiment showed that the curarized muscle can still contract if it is directly stimulated electrically. But if the nerve supplying the muscle is electrically stimulated, then there is no contraction. That was a momentous discovery laying the foundation of the modern pharmacology of drugs binding to targets and receptors. Several poisons and native remedies were studied experimentally, asking *how*?

Table 4.1 lists some of these plants, their clinical effects, and experimental correlates.

The short, 226-page book by Claude Bernard, *An Introduction to the Study of Experimental Medicine*, is considered a classic (Bernard, 1957). L. Bernhard Cohen, in a foreword to its English edition, writes, "The usual definition of a scientific 'classic' is a great work that is venerated, cited, but no longer read. Claude Bernard's book is an exception…" The influence of Bernard on modern pharmacology and drug discovery is all pervasive. Mechanistic explanation of the actions of drug molecules is the most dominant reductionist paradigm even in drug discovery, but history has been forgotten! The human and clinical effects of medicinal plants and poisons started the entire enterprise of drug development and the pharmaceutical industry. Bernard himself had said, "The most useful path for physiology and medicine to follow now is to seek to discover new facts instead of trying to reduce to equations the facts which science already possesses." At least in the early decades of the last century, scientists with medical backgrounds pursued the factual activity of plants in man and searched the mechanisms in animals and tissues. They also gave credit to the practitioners of Asian or folk medicine for the current use

TABLE 4.2 Drug Discovery Paradigm (Vaidya, A.D.B., 2010b)

Medicinal Plant	Clinical Facts	Mechanistic Correlates
Nicotiana tabacum	Stimulant	Nicotinic receptors
Cinchona officinalis	Fever cure	Antiplasmodial
Rauwolfia serpentina	Sedative/antihypertensive	Catecholamine reuptake block
Salix alba	Pain & swelling reduction	COX inhibitor
Ephedra sinensis	Antiasthmatic	Sympathomimetic
Thea sinensis	Stimulant	Increase in CAMP
Catharanthus roseus	WBCs reduction	Microtubules
Erythroxylum coca	Stimulant	Local anesthesia

of the many plants they studied. That was a dominant paradigm that provided targets for the drug actions (Table 4.2).

A huge number of drugs during the last century emerged by testing new chemical activities against the targets as per these mechanisms. The ceaseless activity of medicinal chemists, by minor structural variations on the natural actives, led to an eclipse in finding new facts at the bedside. In addition, the advent of molecular biology and immunology led to hype and hubris for the reductionist paradigm. The developed drugs, based on the mechanisms of plant actions (*vide supra*), are as follows: (1) neuromuscular blockade: tubocurarine, pancuronium, galamine, suxamethonium; (2) anticholinergic: scopalamine, cyclopentlate, iratropium; (3) vitamin K antagonist: dicomarol, warfarin; (4) local anesthetic: procaine, lidocaine; and (5) ACE inhibition: captopril, benazapril, and enalapril. Though the list is much longer than given, the point to be made here is that this mechanisms-directed path led to many "me too" drugs. Eventually, there was a need for a shift in the paradigm.

Rauwolfia serpentina was shown to be an antihypertensive and tranquillizer by Indian scientists Sen and Bose and others (Sen and Bose, 1931). Sen also observed side effects of the plant, namely depression, gynecomastia/galactorrhea, Parkinsonian syndrome, hyperacidity, and nasal congestion. As the active principle of reserpine was isolated, its mechanism was proven to be a depletion of catecholamines by inhibition of their reuptake. A watershed in new drugs occurred for hypertension, depression, Parkinson's disease, prolactinoma, and nasal congestion. A paradigm shift had occurred: Ayurvedic drugs and plants can give therapeutic effects, and that there is much to learn and gain about their side effects, if understood mechanistically (Vaidya, 1979). Unfortunately, there was no organized approach to identify and pursue the clinical facts

of Ayurvedic drug responses to their logical conclusion. The prejudices against Ayurveda among allopaths and fundamentalists of vaidyas (who practiced Ayurveda) have delayed the exploration of Ayurvedic therapeutics for new world drugs. This situation is now changing with the emergence and development of RP, which has caught the attention of several leaders in drug discovery and development. However, the need of triple competence in RP—Ayurveda/traditional medicine, clinical pharmacology, and drug discovery—is a tall demand. This can be partly resolved by a transsystem and transdisciplinary R&D network (Raut and Chorghade, 2014).

THE DEFINITION OF REVERSE PHARMACOLOGY AND ITS DIFFERENT ORIGINS

The need has been felt to look at medical systems, other than modern medicine, in a different perspective, by the leaders of clinical pharmacology like Louis Lasagna and UK Sheth. Lasagna said, "These systems need different type of evidence" (Lasagna, 1999). Sporadic attempts have been made by many groups to study Ayurvedic plants ethnobotanically, phytochemically, experimentally, and clinically. But in the absence of a clear and defined approach the emergent evidence has often been challenged on quantitative grounds or has remained fragmented and not pursued. Alvan Feinstein, a clinical scientist, epidemiologist, and mathematician, wrote in a visionary paper on the limits of quantitation, "A clinician performs an experiment every time he treats a patient. . .each treated patient begins in a baseline state, receives an intervention, and has an outcome—exactly as in an experiment . . .the 'control' comparison comes from the clinicians awareness of similar patients in the past; and the goal of the 'experiment' is to repeat (or exceed) the best outcomes achieved with those previous patients." (Feinstein, 1994). During a stay at Yale, of one of the authors (ADBV) had several discussions on clinical research with Feinstein, Robert Levine, Arnold Eisenfeld, Mel Van Woert, and Lewis Thomas. A vague sense of direction then emerged, presaging RP. The stress by Feinstein and Levine on starting research from the clinic—a bedside to bench path—made a major impact (Vaidya, 2013).

After much deliberation, an acceptable and pragmatic definition has emerged for RP, which is the science of documenting clinical/experiential hits with basal and postintervention data, and then by relevant trans-system exploratory studies (in vitro and in vivo) of these hits to develop leads. Positive leads are then investigated at different levels of biological organization, experimentally, and clinically as drug candidates.

The scope of RP is immense: (1) to evaluate clinically the evidence of safety, efficacy, and quality of drugs/plants used in the TSM; (2) to discover new drugs from natural products (NPs) already in use by humans; (3) to find new clinical facts and bedside biodynamic phenomena that may lead to new

TABLE 4.3 The Starting Points of Reverse Pharmacology

Systems and Domains	New Approaches	Clinical Research
Ayurveda/siddha	Ayurvedic epidemiology	Anecdotal cases
Traditional Chinese medicine	Observational therapeutics	Case reports
Unani medicine	Systems ayurveda	Case series
Homeopathy	Phytochemistry	Retrospective surveys
Kampo medicine	Golden triangle	Retrolective studies
Tribal medicine	Ayurvedic biology	Prospective Studies
Modern medicine	Phytopharmacolgy	Open clinical trials
Ethnobotany	Ayurgenomics	Controlled trials
Vriksha/prani ayurveda	Molecular botany	Multicentric trials
Nutraceutics	Reverse nutraceutics	Meta-analyses/ Cochrane

insights in human biology; (4) to overcome the current costly, drawn out and attritive process of drug discovery/development; and (5) to complement the extant process by novel phyto-actives as chemical scaffolds for NCEs.

RP need not merely be a linear process or unimodal in its resourcing. The origins of RP can also be from the diverse and rich big data of traditional/modern literature, ethnobotanical, phytochemical, experimental, clinical, and anecdotal cases. Table 4.3 lists systems and domains, new approaches and areas of clinical research. There is a vast scope of exploring RP by a judicious combination of systems of origin, new approaches, and areas of clinical research.

AYURVEDIC PHARMACOEPIDEMIOLOGY AND OBSERVATIONAL THERAPEUTICS

The main domains of RP driven drug discovery are (1) experiential, (2) exploratory, and (3) experimental. These three domains are rather exclusive mutually, not sequential. RP is a circular model with enrichment interconnecting feedbacks. However, experience mostly forms the first platform of RP. The experiential domain of RP would cover Pharmacoepidemiology-resourced information, hints from clinical notes and classical literature, and hits from observational therapeutics as well as from single case studies/case series. Experiential domain would source information coming from unconventional rationales of traditional medicine, from novel clinical observations to serendipitous findings.

Ayurvedic Pharmacoepidemiology

The discipline of pharmacoepidemiology (Strom, 1989) is the outcome of an integrative approach of clinical pharmacology and epidemiology. During the major multiinstitutional national session of the Council for Scientific and Industrial Research sponsored by New Millennium Indian Technology Leadership Initiative (CSIR-NMITLI) in arthritis, diabetes, and hepatitis for developing globally competitive herbal drugs inspired from Ayurvedic heritage, the idea of Ayurvedic pharmacoepidemiology was proposed by Dr. Rama Vaidya (Vaidya et al., 2003). Ayurvedic pharmacoepidemiology would require a collaboration among vaidya scientists, clinical pharmacologists, and epidemiologists.

Ayurvedic pharmacoepidemiology (AyPE) is defined as the "study of the usage, acceptability, efficacy, safety, complementarities, and cost-effectiveness of Ayurvedic drugs in a large number of people." It includes Ayurvedic prescription audits, registration of Ayurvedic drugs utilization, population pharmacodynamics/kinetics, and documentation of untoward or unexpected beneficial effects of Ayurvedic drugs. AyPE aims to study Ayurvedic therapies in various aspects such as the extent of use, level of efficacy, nature of safety, cost-effectiveness, drug interactions, and rationality as per the indications and ingredients of the drugs (Vaidya et al., 2003).

With a global increase in the use of herbal and traditional therapeutics (herbal supplement sales are expected to hit $93.15 billion by 2015: Report [Internet]) the Pharmacoepidemiology of Traditional medicine will become more relevant. In the Indian context, where Ayurveda is deeply rooted in the community at large, Ayurvedic pharmacoepidemiology becomes quite relevant to healthcare. Such an endeavor can be useful in many ways: (1) prevalence and patterns of usage of Ayurvedic drugs; (2) records of field safety for rational therapeutics and precautions; (3) establishing a national drug policy on the pharmaco-economics of Ayurveda; (4) detection of drug interactions due to concomitant intersystem utilization of drugs; and (5) discovery of novel, beneficial effects for developing new drugs.

Two doctoral (PhD) programs were initiated on Ayurvedic pharmacoepidemiology in arthritis and in diabetes. Whereas the program on arthritis has been submitted to the university (Tillu, 2015), the program in diabetes has also progressed well. The review of the literature and current status of indigenous antidiabetic drugs was published in a textbook on diabetes mellitus (Vaidya et al., 2014). A detailed analysis of marketed Ayurvedic antidiabetic formulations for their brand names, dosage forms, ingredients, composition, dosages schedule, and precautions and package insert has provided interesting data on Ayurvedic-marketed products in the Mumbai region of India (Nabar et al., 2013) During a drug utilization study at a healthcare medical camp and at a tertiary care center it became evident that the majority of patients do use indigenous and conventional antidiabetic

drugs concomitantly. These can be with or without the information of the treating physician (unpublished data). This raises the question about synergistic or antagonistic activities of drug interactions. The concern for drug interaction got substantiated when in one volunteer the bioavailability of metformin was reduced by Ayurvedic powder (Puranik et al., 2014). The KAP survey (knowledge, attitude, and practice) on the management of diabetes and arthritis is currently in progress on patients as well as for traditional Ayurvedic practitioners.

Observational Therapeutics

Observational therapeutics can be defined as an observational study that carefully documents the therapeutic outcomes and novel biodynamic effects of interventions from conventionally practiced therapy. Several modern drug discoveries have their roots in careful follow-up of chance observations in the field, in patients, or in laboratories. The ancient classical literature of Ayurveda, clinical notes, and observations of Ayurvedic experts and recent clinical use provide a goldmine for further drug research through observational therapeutics (Sen and Bose, 1931; Raut, 2010; Pade, 1973; Aushadhi Baad, 1974; Desai, 1928; Vaidya, 1925). There is an urgent need for a strategic plan to organize observational therapeutics at Ayurvedic teaching hospitals so that the experiential knowledge and wisdom of traditional medicine can help to discover and develop Ayurveda-inspired new drugs or new phytoactive chemical scaffolds.

Dr. Rama Vaidya, in her thought leadership article on *Observational Therapeutics: Scope, Challenges and Organization*, has given key messages relevant to finding new approaches for traditional medicine-inspired new drug discovery (Vaidya, 2011). These are as follows:

- "Exclusive hierarchy of randomized controlled trials, along with evidence-based medicine, has largely eclipsed the significance of even valuable observational studies."
- "Observational studies could be judged on the basis of the validity of causal associations on well-defined criteria such as dose-response relationship, temporal sequence, and biological plausibility."
- "Inspirational impact of new hits and leads has to be shared at the institutional morning reports, grand rounds, continued medical education, and widely read journals."
- "A judicious and economical usage of advanced markers necessitates robust thinking of biological plausibility and rational understanding of *Dravya-Guna-Vidnyan* (Ayurvedic pharmacology)."

Ayurvedic pharmacoepidemiology essentially makes an assessment of the field reality and community practices of Ayurveda therapeutics and

principles. Such an endeavor should establish communication with research methodologies to bring scientific credibility to these practices and help correct wherever is needed to rationalize the community practices. Observational therapeutics on the other hand could be more focused to the clinical setups. This has the inbuilt advantage of knowledge and wisdom of practicing physicians. Such an endeavor has the potential to identify emerging hits and leads from clinical practices for new drug discovery and development or even for repurposing traditional drugs for new indications.

CLINICAL STUDY DESIGNS AND PARA-CLINICAL MODELS IN REVERSE PHARMACOLOGY

There are three stages of RP: (1) experiential, (2) exploratory, and (3) experimental. The study designs do not follow the conventional Phases 1−3 that's mandatory for NCEs. This is primarily so because in RP one starts at the bedside first. The physician in charge of the patient carries out the experiential studies in the regular clinical practice settings. The treatment given is a standard practice. The added elements are essentially the records of the baseline clinical and laboratory findings/markers that define the therapeutic or adverse response. A Reverse pharmacologist joins the caretaker physician just to ensure that meticulous attention to the minutest details is a mindful and ceaseless activity. For example, in an experiential study of malaria a remedy was given in a dose and form as per the routine. But the temperature and symptoms profile were meticulously recorded and graded. The malarial parasite (MP) count was carried out basally and followed up. The data suggested early response to the crude plant paste from the leaves of a single tree of *Nyctanthes arbor-tristis* as compared to the natural course of clinical malaria (Karnik et al., 2008). The sample size was large and the Ayurvedic literature too suggested usage and activity against malaria. An experiential stage needs to stress good clinical service practice. Whether these can be called trials is a moot point. In a therapeutic setting, the physician has the patient's trust and he or she knows how to monitor them.

During the exploratory studies—human or in vitro/in vivo models—the choice of methods and design has to be relevant to the earlier observations in Stage 1. The designs can involve $N = 1$ studies, human pharmacology (noninvasive methods) for dynamics, dose-searching/dose-finding/dose-optimizing studies with markers of response, pharmacokinetics (if an assay is available), sequential trial design, and an open comparative or cross-over trials can be scheduled, as per the individual intervention and the indication. The standard clinical pharmacology techniques and design will have to be modified and adopted, without the orthodox Phase 1−3 approaches. For example, the plant studied experientially for malaria was subjected to exploratory studies with more frequent temperature (inner ear) measurements.

Besides the MP counts, a PCR for the plasmodial DNA was conducted for the parasite clearance. The markers of severity—inflammatory cytokines such as TNF-α and ILβ1—were measured basally and serially. A disease modifying activity was observed with a faster amelioration of symptoms (Godse, 2004). The plant was extracted with rigorous protocols and active fractions were identified against *Plasmodium falciparum* strains: resistant and sensitive. The fractions were studied in several other in vitro models. The point to notice is that individualization of the designs and models is a crucial dimension of RP.

The experimental and final clinical stage have the rigor that can match that with any NCEs in terms of the proof of the concept of efficacy, safety, and quality. But again only the relevant science is brought to the fore as the usage safety and therapeutic indications were known to start with. The mechanistic studies can be under taken as paraclinical rather than as preclinical studies. The clinical trial designs have to consider the Ayurvedic *pramanas* (*apta, pratyaksha, anumana, upamana*, and *yukti*), besides the statistical considerations; Ayurvedic statistics are being developed for this purpose (Vaidya, 2007). The clinical trials will have robust markers of efficacy and safety as per the interventions and indications. Instead of double-blind trials, double-vision trials are proposed in RP. The phrase "double vision" implies a conscious consideration of factors that influence drug response. The Ayurvedic modifiers of the therapeutic response are to be factored-in rather than lumped-out as confounding variables to be equally distributed in the control and test groups.

The entire thrust has to develop analogue models of what clinical drug actions have been documented. The bedside-to-bench path has this advantage. The data then can be taken back to the bedside for a drug with much more confidence than we do with NCEs as new drugs. The withdrawal of blockbuster drugs has taught us the lesson to go much earlier in humans (Patwardhan, 2008). Sir John Vane, Nobel Laureate, said,

"[R]egrettably there was ample evidence that the time taken from discovery to marketing was lengthening (8−10 years)...but I was aware of at least one instance where it had taken more than 17 years. Clearly there is a need, to conduct the early evaluation of drugs in humans more effectively and more readily."

RP can provide such a new paradigm. Even a pioneer like Bradford Hill said,

"Such personal observations of a handful of patients, acutely made and accurately recorded by the masters of clinical medicine, have been and will continue to be, fundamental to the progress of medicine...We were to use a new drug upon one proven case of acute leukemia and the patient made an immediate and indisputable recovery, should we not have a result of the most profound importance."

AYURVEDA-INSPIRED HITS AND LEADS FOR NEW DRUG DISCOVERIES

Inspirational elements of Ayurveda are driven from its robust fundamental principles and long-standing social acceptance. The core fundamental principles of *yatha-pinde-tatha-brahmande* (microcosm−macrocosm−continuum) and *sharir-satva-atman* (body-mind-spirit) bespeaks of its holistic and integrative approach. Whereas *panchamahabhuta-tridosha-triguna* (five basic elements-three pathophysiological attributes-three psychological attributes) and *hetu-linga-aushadhi* (cause-manifestations-management) indicate a comprehensive and systems theory approach of Ayurveda. The utility and applicability of this traditional healthcare heritage is evident through its live classical texts with editions and commentaries, several therapeutic compendia, clinical notes of masters, and a large community usage in the Indian and south Asian populace.

Sarpagandha (*Rauwalfia serpentina*) was the first Ayurveda-inspired new drug discovery that made a global impact on modern pharmacotherapeutics of hypertension. It was an early example of antecedent preorganized RP inspired from Ayurvedic therapeutics. A Sarpagandha formulation-called Pagal Buti was popularly used by traditional practitioners of Ayurveda for psychiatric indications. The significance of *R. serpentina* as an antihypertensive was appreciated by Vaidya Kaviraj Gananath Sen along with Kartick Chander Bose in the early 1930s (Sen and Bose, 1931). The study demonstrated a reduction in blood pressure and a tranquilizing effect. This was an experience-driven clinical hit. Subsequent studies by Siddiqui and Siddiqui and others were on the phytochemical analysis (Jain and Murthy, 2009). A historical 1949 paper in the British Heart Journal by Rustom Jal Vakil (Vakil, 1949) summed up his 10 years of study with added opinions of some 50 other physicians with rauwolfia in hypertension. The publication led to the drug finally being brought to Western awareness (Gupta, 2002). CIBA scientists isolated reserpine and a global antihypertensive drug was made available.

Arogyavardhini Vati is one of the popular Ayurvedic formulations classically indicated for diverse clinical conditions. However, it is most commonly used for jaundice, along with Punarnavadi Kwath by Ayurveda practitioners of western India. Inspired from this clinical usage, an open-label clinical study was conducted that showed safety and efficacy of this combination in acute viral hepatitis (Antarkar et al., 1978). Subsequently, a well-designed, double-blind, placebo-controlled clinical study was conducted of only Arogyavardhini Vati in acute viral hepatitis in the mid-1970s. This 14-day clinical study in 38 subjects provided convincing evidence for its efficacy in early reduction of liver transaminases as well as clinical morbidity scores (Antarkar et al., 1980). Further from this complex herbo-mineral formulation of Arogyavardhini; Kutaki (Picrorriza kurroa) a 50% content of it was

optimized for the phytoactives; picrocide 1 & 2. Such a standardized Kutaki was subjected to placebo-controlled trial that also demonstrated efficacy in viral hepatitis (Vaidya et al., 1996). These clinical hits and leads were studied further in experimental models of liver injury (Shetty et al., 2010).

Atmagupta (*Mucuna pruriens*) contains formulations that are often used in Ayurveda for improving general vitality and as an aphrodisiac. However, repurposing its use for Parkinson's disease (PD) was based on a phytochemical analogy struck by one of the authors (ADBV). PD is a disease of dopamine deficiency in the corpus striatum. *M. pruriens* contains high levels of L-Dopa (Damodaran and Ramaswamy, 1937). With this literature-driven hit, a clinical study of *M. pruriens* in PD was planned. This exploratory clinical study demonstrated the efficacy of *M. pruriens* in PD with concomitant displays of plasma levels of L-dopa. This single open label, but well-organized study, provided an optimized lead (Vaidya et al., 1978). Recently, in a comparative cross-over clinical study with L-dopa-carbidopa combination, *M. pruriens* has shown better safety and efficacy (Katzenschlager et al., 2004). Compliant formulation with reduced bulk and improved palatability remains the main challenge. There are basic studies showing anti-Parkinson's effects with L-Dopa−free extracts of *M. pruriens*.

Amruta (*Tinospora cordifolia*) is considered a *rasayana* (rejuvenative/reparative) plant in Ayurveda. Initial investigation of this plant was driven by the hint of a conceptual correlation of rasayana with that of immunomodulation (Dahanukar et al., 1988). This plant has been extensively studied experimentally as well as clinically for diverse pharmacological activities, namely, immunomodulatory, antiinflammatory, antiarthritic, antipyretic, hepatoprotective, antidiabetic, antispasmodic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, and antineoplastic activities (Upadhyay et al., 2010; Panchabhai et al., 2008). Several phytoconstituents have been isolated and characterized including seven immunomodulatory compounds (11-hydroxymustakone, *N*-methyl-2-pyrrolidone, *N*-formylannonain, cordifolioside A, magnoflorine, tinocordiside, syringin) characterized from different parts of this plant (Sharma et al., 2012). However, whole plant extracts are used clinically and are commercially available.

Commiphora wightii (Guggulu) is an illustrious plant medicine from Ayurveda that has gone through all three phases of RP: gaining hits from traditional literature, developing into leads through clinical, animal and laboratory studies and eventually became a natural drug available globally for the specific indication of dyslipidemia. However, in a study published in JAMA in 2003 on Guggulipid for the treatment of hypercholesterolemia (a randomized controlled trial), sponsored by the NIH, the results were negative (Szapary et al., 2003). This single negative paper had an adverse impact on the Guggulipid market and also on the interest of scientists and funding agencies in Guggulu. However, at the same time the molecular mechanism of guggulsterones was demonstrated: Guggulu had lipid-lowering activity

through antagonizing Farnesoid X receptor (Urizar et al., 2002). Scientists with clinical conviction continued to work on Guggulu/guggulsterones. Over the last few years guggulsterones/Guggulu components are being experimentally explored for diverse pathological conditions, in particular different cancers, inflammatory colon conditions, ophthalmic and ear inflammatory conditions, synovitis, hepatitis, crystal arthropathy, atherosclerosis, endothelial dysfunction, cardiac dysfunction, insulin resistance, metabolic disorders, bacterial infections, skin aging, and so on. While the current interface of Guggulu with basic sciences is opening windows, the true picture is still eluding scientists (Raut and Mertia, 2012).

Several such Ayurvedic plants/formulations have been investigated, which provide hits and leads with prospective new drug discovery/NPs development. Some of the selected plants/formulations are tabulated (Table 4.4).

A clinical research model taking into account the fundamental principles of traditional medicine would appreciate the therapeutic approach of the traditional medical system. At the same time the application of the methods of relevant drug discovery sciences would be most appropriate. The establishment of such evidence would rationalize traditional medical practices. An RP approach is the most suitable to establish such evidence (Vaidya, 2010a). The untapped potential for the new drug discovery from RP and in traditional medicine is immense.

DISCOVERY OF NATURAL PRODUCTS-BASED NEW CHEMICAL ENTITIES VIA CHEMISTRY AND CHEMICAL BIOLOGY TECHNOLOGIES

Introduction and Background

The natural world is a source for inspiration for chemists and biologists, and NPs have long been a source for novel molecules of broad utility. The exquisite and varied architecture of NPs provides a rich palette for discovery. They can be considered "prevalidated by nature," having been optimized for interaction with biological macromolecules through evolutionary selection processes. Besides being useful in their own right, these compounds represent a diverse source of novel, active agents that can serve as leads/scaffolds for elaboration into NCEs for a multitude of explorations.

Embedded in these NPs are a number of diverse, chiral functional groups that are potential sites for protein interactions. Biologically active NPs generally have frameworks with rigid and complex ring structures and precise stereochemical features. This ensures that derivatives from these starting compounds are likely to yield leads with higher specificity and affinity.

TABLE 4.4 Ayurveda-Inspired and Reverse Pharmacology-Driven Hits and Leads

Plants/ Formulations	Indication/Hit	Activity/Lead	Impact
AmrutBhallatak (Raut et al., 2007; Raut et al., 2013) *Semecarpus anacardium* and *Tinospora cordifolia*	Osteoarthritis	Chondroprotection	Disease modifying antiosteoarthritic
Parijat (Godse, 2004) *Nyctanthes arbor-tristis*	Malaria	Antiparasite	Disease modifying
		Anticytokine	Isolation of bioactive fraction
Mamejawa (Vaidya, 2007) *Enicostema littorale*	Type 2 DM	Lipemic control	Prevention of complications
		Antioxidant	
		DNA protection	
		Glycemic control	
Panchavalkal (Joshi et al., 2007) (five barks)	Wound healing	Vaginal infections	Ayurvedic topical products in market
	Leucorrhoea	Burns wounds	
Ashoka (Shringi, 2000) *Saraca asoca*	Menorrhagia	Ovulatory dysfunctional uterine bleeding	Menorrhagia subset identification
Haridra (Hastak et al., 1997; Joshi et al., 2011) *Curcuma longa*	Oral-submucus fibrosis	Micronuclei reduction in oral smear	Anticancer studies, studies in neurodegenerative disorders
	Cervical precancer	Reduction in persistent cervical precancer	
	Antiaging	Hippocampal neurogenesis	
Shunthi (Altman and Marcussen, 2001; White, 2007) *Zingiber officinalis*	Nausea, vomiting, arthritis	Antiemetic, antiinflammatory, and antiarthritic	High evidence of efficacy levels

Ashok Vaidya et al., in their seminal studies (Patwardhan et al., 2008; Vaidya, 2014), have demonstrated that many modern drugs and agrochemicals have their origin in ethnopharmacology and traditional medicine. Ayurvedic and traditional Chinese systems are living "great traditions." Traditional knowledge and experiential databases can provide new functional leads to reduce time, expense, and toxicity—the three main hurdles. Thus, the normal discovery course of "lab to utility" actually becomes "utility to lab"—a true reversed design approach.

The enthusiasm for NP discovery is dampened by difficult syntheses. A significant disadvantage of NPs, with the exception of those derived from fermentation, is the draconian organic synthesis/medicinal chemistry effort required for commercialization or future functionalization. In many cases, the NP is not available in sufficient quantities for various biological assays, thereby limiting their exploration.

Chemical space must be expanded efficiently. The ability to easily access new chemical space is a major challenge for discovery chemists. Although advances in parallel synthesis/combinatorial chemistry and diversity-oriented synthesis have made great strides towards expanding the accessibility of synthetic compounds that have high levels of diversity (including stereochemical, shape, and bond connectivity), there is room for further improvement. The diversity expansion inherent in transformations that mimic the metabolism of small molecules and NPs can provide a new direction. Simple and well-controlled oxidation, halogenation, and alkylation reactions can afford compounds that have unique physicochemical and biological properties. Indeed, Muller has opined that such biotransformations are the source of remarkable levels of diversity in NPs and investigational agents (Müller, 2004).

Chemists prefer known and reliable chemistry. Despite possessing an enviable armamentarium of techniques and methodologies in organic synthesis, chemists often prefer to focus on the simplest, most robust (and therefore commonly used) transformations. This is driven by premium demands on efficiency and creativity. As a result, much of the emphasis in industrial research involves the study of molecules that can easily be made, rather than on those agents that properly address the question but require significant synthetic innovation, isolation, and synthesis of indigenous NPs and their derivatives. Several plants have been used in traditional Ayurveda and Siddha medicine; the NPs have demonstrated or have been ascribed medicinal value after years of experience. The latest techniques in systems biology and RP can be used to isolate and characterize the bioactive agents and generate key scaffolds with chirality. These architectures have hitherto not been used in discovery chemistry. The need of the hour is to derive inspiration from the wisdom of Mother Nature to reconfigure products into chemical hybrid "molecular legos" and to screen the deck of diverse compounds against targets. Recently, the concept of hybrid molecules has gained currency. Coupling of diverse molecules such as artemisinin and chloroquine with

vitamin K3 have generated new molecules with effectiveness in oncology. In addition to diversifying the structure of single molecules, the properties of several NPs can be modulated by such hybrids.

Current chemical investigations center on: (1) expansion of the already known activities of NPs derived from RP by selective functionalization, generation, and derivatization of privileged structures and conducting selective studies; (2) designed organic synthesis of high-recognition libraries focused on specific biological targets; and (3) synthesis of building blocks/scaffolds/high-value intermediates.

We have a repository of molecules that contains:

- Six hundred scaffolds/building blocks derived from purification of extracts from the top 100 medicinally useful plants that have been used in Ayurveda, yoga, Yunani, Siddha, and homeopathy.
- Nearly 1000 pharmaceuticals that are currently available free of patents. These allopathic drugs range from acyclovir to ciprofloxacin to roxithromycin and sidenafil.
- Nearly 2500 intermediates from which the above drugs have been synthesized by nonpatent infringing processes. These molecules are drug-like but, on the whole, have not been tested in the West.

Chemists are actively engaged in the development and streamlining of chemical technologies that enable the transformation of NPs and their derivatives from this repository directly to NP-derived new chemical entities. A collection of existing and novel oxidation approaches that are capable of robustly and predictably converting functionality typically found in the specific classes of NPs are used. These oxidations include metalloporphyrin-mediated oxidations, hepatocyte oxidations, salen-based oxidants, and halogenation reactions. Key automated platform technologies to streamline the application of these oxidation chemistries en masse to NP collections are being deployed. Such technologies include flow chemistry (Ley, Jones), development of new resin-bound reagents (Ley), microwave chemistry (Jones), autopurification (Jones), and automated structure elucidation (Jas and Kirschning, 2003; Sedelmeier et al., 2009; Gedye et al., 1986; Caddick, 1995; Kappe, 2004; Torregrossa et al., 2006; Collins, 2010; de la Hoz et al., 2005; Huber and Jones, 1992; Jones and Chapman, 1993).

Isolation of Natural Products from Plant Species with Established Indian Folk Medicine Properties

Chorghade's discovery approach utilizes biologically active NPs integrated with an established, efficient synthesis toolbox. Our unique access to discovery and development quantities of isolated NPs with known biological activity is an asset. We use a modified bioassay-guided fractionation approach wherein a high throughput separation chemistry approach (using the parallel

high-performance liquid chromatography (HPLC) "Sepbox" technology, Sepiatec, Inc.) is coupled with cell-based bioassays to detect and identify putative compounds that offer promise of bioactivity. A significant advantage is that in addition to fast separation and isolation of active fractions, the cell-based phenotypic effects will allow emphasis on bioactive samples.

Some active fractions (possessing other putative biological activities) might be eliminated due to their lack of effect in the assays coupled to fractionation; subsequent testing of these fractions in other phenotypic assays and follow-up of structural identification work will isolate the full spectrum of these bioactive compounds.

Sample Collection

Plant sample collection is an important aspect of NP drug discovery, since the secondary metabolites are formed under certain macro and/or micro environments.

Fractionation

NP drug discovery is always a challenge. The "Sepbox" provides a novel high-throughput approach to support fractionation and subsequent identification of compounds within the fraction. The Sepbox concept is based on a patented combination of HPLC and solid phase extraction (SPE). Using two-dimensional separation, the recovery rate for both polar and nonpolar substances is usually above 90 percent. Using an automated and highly reproducible process, one extract can be completely separated in one day. The pure individual components are solubilized in suitable solvents and collected in microtiter plates or vials. The compounds collected in microtiter plates can be used directly for assaying or can be used as a master plate for making HTS assay plates. Up to 600 fractions with a very high yield of pure compounds can be efficiently obtained to be used in subsequent screenings.

Fractions are evaluated contemporaneously via HPLC/UV/LC-MS and LC-MS-MS detection for chemical composition. The usual spectroscopic tools, FTIR, NMR, CMR, and MALDI-TOF, are used for structure elucidation and characterization. Chiral HPLC ascertains the stereochemical configuration with additional derivatization, as needed.

Design and Synthesis of Natural Product Analogs (McNamara et al., 2002; Sanchez-Martin et al., 2004; Baxendale et al., 2006)

Antimalarial lactones: Secondary metabolites isolated from *Cordeceps militaris* have received attention due to their unique structures and specific biological activities (Fig. 4.2). Compound A was isolated as a white solid from *C. militaris* BCC 2816; the structure was elucidated and the stereochemistry confirmed by spectral data and X-ray crystallographic analysis. Some natural

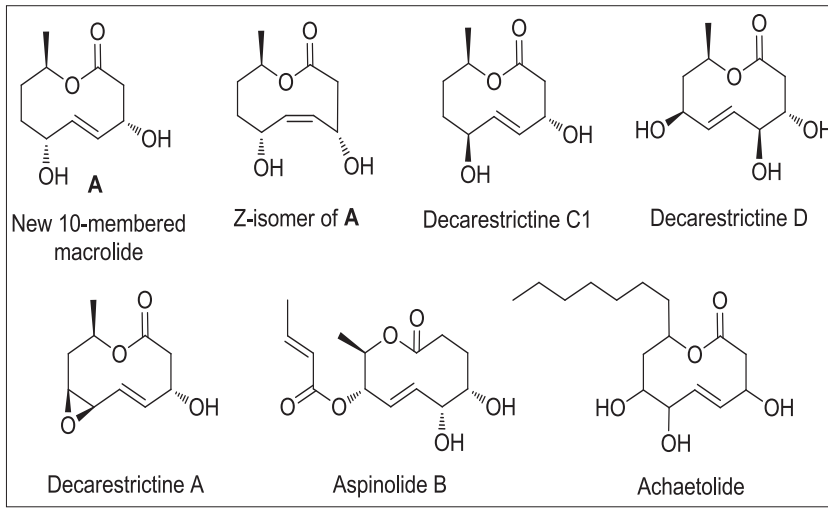
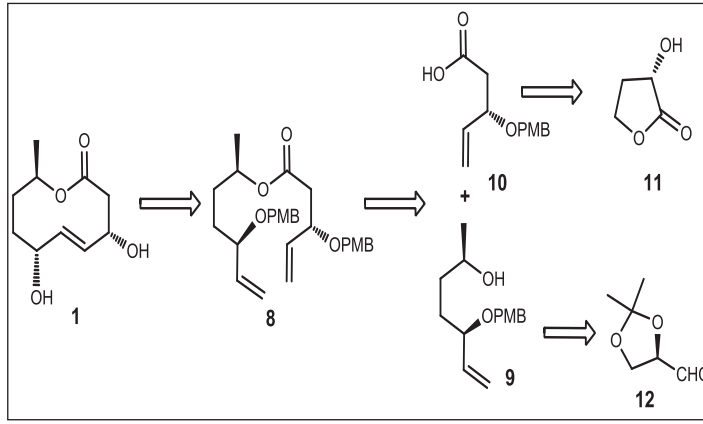


FIGURE 4.2 Natural products derived from *Cordyceps militaris*.



SCHEME 4.1 Retrosynthesis of compound A.

nonenolides possess chiral centers on both sides of a double bond. These have a variety of biological activities ranging from antidiabetic to cholesterol lowering to antileishmanial. The compounds enumerated above could serve as scaffolds that could be elaborated into a variety of applications.

In a collaborative program, with Professors Grubbs and Goddard (California Institute of Technology) on the synthesis of natural lactones that employs ring-closing metathesis (RCM) as key step, Chorghade et al. Mohapatra et al. (2007) devised a stereoselective synthesis of nonenolide (Scheme 4.1). Conventional wisdom held that the stereochemistry of

ring-closing metatheses could not be accurately predicted for 8-, 9-, and 10-membered ring lactones. The macrolactonization step relies on a RCM on a diolefinic ester. Strategic bond disconnection in ester 8 leads to chiral, nonracemic fragments 9 and 10 that could be derived from (S)-α-hydroxy-γ-butyrolactone 11 and 1, 2-*O*-isopropylidene (D)-glyceraldehyde 12, respectively. Ester 8 was subjected to RCM reactions in two different sequences: First, the RCM reaction was conducted on the protected species; deprotection yielded a preponderance of the E-isomer. Deprotection, followed by RCM, yielded a preponderance of the Z-isomer. Extensive density functional theory calculations conclusively identified a pivotal role for the protecting groups in directing the stereochemistry of ring closure. The work was instrumental in the elucidation of some specific rules about stereochemical requirements for olefin metathesis.

REVERSE PHARMACOLOGY AND NEW DOMAINS IN LIFE SCIENCES

Once the active NCEs have been isolated from a NP after meticulous RP, many synthetic and analytical techniques can be explored to expand the scope with the naturally available chirality and diversity of these molecules. Extrapolations to pharmaceuticals, agrochemicals, and cosmoceuticals are feasible. Representative new technologies are enumerated below.

Automated Oxidation Chemistry for Diversified Analogues

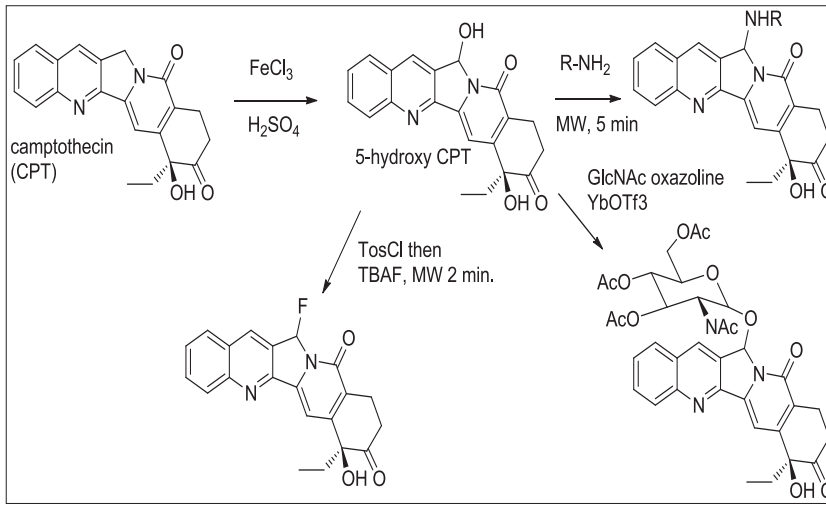
Chorghade, Dolphin, and colleagues (Andersen et al., 1994; Chorghade et al., 1996a, b; Hill et al., 1996) have developed novel sterically protected and electronically activated porphyrin mediated catalytic oxidation of sophisticated molecular entities and have designed numerous practical and efficacious methods of synthesizing porphyrins with halogens at the ortho-aryl and also the pyrrole positions and central metal atoms such as magnesium, manganese, and ruthenium in the macrocycle. Traditional porphyrins suffer very rapid oxidative degradation and dimerization; the catalytic turnovers and reaction rates are very low. These catalysts have turnover numbers in excess of 100,000 and are extremely stable.

The methodologies have been used to achieve epoxidation, hydroxylation, and *N*-demethylation on numerous targets including functionalized NP substrates; the N and S oxides are also obtained. The oxidation procedure is extremely facile as compared to the biochemical and enzymatic processes. Exogenous cooxidants such as iodosobenzene, cumene hydroperoxide, hydrogen peroxide, or sodium hypochlorite were used; substrates were stirred for one to six hours at ambient temperatures. Products were separated by a combination of HPLC and preparative Thin layer chromatography. A library of compounds, when subjected to porphyrin-mediated oxidation, yield a

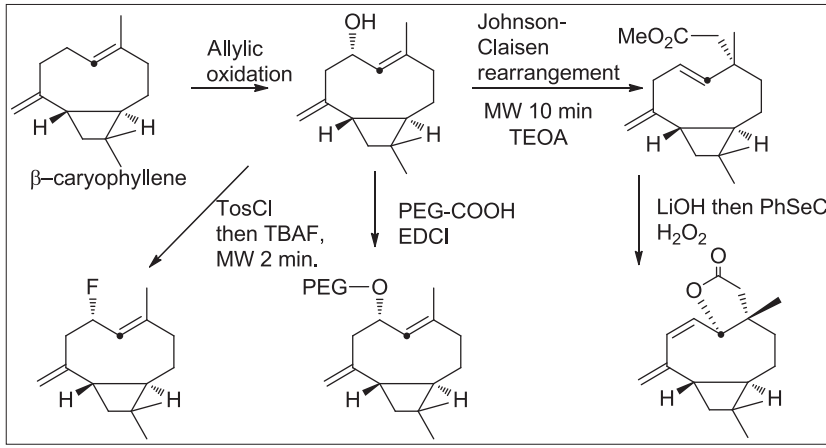
substantially larger number of compounds. This then provides new compounds that are more polar, water soluble, and contain handles for further derivatization. RP-based new phytoactives can be subjected to the methodologies (*vide supra*) to expand the library of molecules with drug-like activities, as earlier shown, clinically, with parent molecules.

Transformations in this platform have two ultimate outcomes: (1) modification of lipophilicity: small changes in lipophilicity of a lead molecule provide compounds with significantly different physicochemical and biological properties and (2) enabling rapid compound follow-up: a significant consideration is the amenability of the scaffold for rapid evolution. In addition, installation of a functional group easily transformed into a radiolabel provides rapid inroads to crucial imaging experiments. The likely conversion of rolipram into hydroxylated analogs via oxidation chemistry could make these molecules susceptible to a tosylation/fluorination sequence for installation of ^{18}F for PET or ^{123}I for SPECT imaging.

Similar strategies can be applied to NPs with established biological targets to probe pathways and potentially uncover new analogs with refined properties. Consider the antitumor agent alkaloid camptothecin, isolated from *Camptotheca acuminata*. Targets of this storied agent include the regulatory enzyme topoisomerase I and hypoxia inducible factor 1 [HIF-1α]. This compound exists in the hemiaminal form, and Jones (Torregrossa et al., 2006; Collins, 2010; de la Hoz et al., 2005; Huber and Jones, 1992; Jones and Chapman, 1993; Jones and Mathews, 1997; LaBeaume et al., 2010b) has shown that by using microwave-mediated methods, a facile conversion to derivatives of 5-amino CPT can be effected (Jones). A 5-fluoroethyl derivative shows superior HIF-1α inhibition than CPT, warranting an in-depth synthesis and screening program. Additionally, microwave-mediated fluorination methods would seem to be suited to formation of 5-F and, using radiolabeled fluoride, the corresponding ^{18}F derivative to permit PET imaging for distribution studies. DNA repair enzymes are a target of CPT; another option could include conversion to GlcNAc and other carbohydrate derivatives using established glycosylation coupling chemistries (Ma et al., 2009; Dong et al., 2008; Kallmerten and Jones, 2010; Labeaume et al., 2010a) (Scheme 4.2). Myriad other NP platforms are amenable to selective oxidation chemistry (LaBeaume et al., 2009; Peddibhotla et al., 2007). The bicyclic sesquiterpene caryophyllene, recently identified as a ligand for the cannabinoid CB2 receptor, has folk medicinal applications as an analgesic and antiinflammatory agent. Selective allylic oxidation would allow conversion to a number of derivatives of increasing complexity and differing lipophilicity and transport properties. This could include conversion to a PEGylated analogue, fluorination in order to study metabolic profiles and with labeled [^{18}F, ^{123}I] versions, molecular imaging (Scheme 4.3). Jones employed microwave-mediated Johnson-orthoester Claisen rearrangement on the derivative (Jones et al., 1993). The resulting ester, when subjected to



SCHEME 4.2 Hydroxylated CPT derivatives as platforms to substituted analogues and probes of CPT function.



SCHEME 4.3 Selective oxidative strategies en route to beta-caryophyllene derivatives.

selenolactonization, yielded the cyclic lactone derivatives. Given the abundance of cyclic lactones in terpene derived NPs, this could open up new avenues of research in their relevant biochemical pathways and targets.

REVERSE PHARMACOLOGY APPROACH TO GPCR-FOCUSED DRUG DISCOVERY

Bennett et al. (Bennett et al., 2013), in Molecular Pharmacology, describes how RP, enabled by Heptares Therapeutics StaR(R) technology, can be

applied to and accelerate GPCR-based drug discovery. The authors studied isolated GPCRs locked in conformations that correspond to agonist or antagonist pharmacology, and elucidated 3D structures. These StaRs and structures were used to select and design compounds with specific pharmacologies such as inverse agonist, partial agonist or full agonist, based on their ability to bind differentially to the agonist and antagonist StaRs.

Finally, new techniques are also used for the standardization and characterization of herbal remedies that are sold as supplements and are contaminated with unlisted ingredients that could pose health risks to consumers. Scientists will now use advanced DNA testing to authenticate all of the plants that are used in its store-brand herbal supplements, and extensively test the products for common allergens like tree nuts, soy, and wheat.

Reverse Pharmacology and Novel Biodynamic Actions

Bedside observations of patients or field observations of people have often identified novel effects of foods, plants, and drugs. The majority of such astute observations are often serendipitous and frequently not followed up scientifically. The organized system of Ayurveda and TCM, as practiced currently, offer unprecedented opportunities for both serendipitous as well as planned records of novel drug effects. Several disciplines of life sciences can be traced back to their roots in novel human biodynamic actions. RP is a multisystem path for a scientific pursuit of such actions. For example, the trichomes of the fruits of *Mucuna pruriens* induces intense itching when in contact with skin. We had studied the scanning electronic microscopic view of the trichome (Fig. 4.3). The trichome was a hollow tube with miniscule reversed hooks on its surface. This structure opened up the field of investigations into the mechanism of pruritus vis-à-vis mast cell degranulation. This would probably enhance the field of mechanistic understanding of allergic urticaria and itching. Another plant, *Gymnema sylvestre*, relieves trichome-induced itching (Vaidya, 1910). RP of this plant would open up the potential for novel phytoactive antipruritic agents.

Ayurveda has given central importance for health on the functional competence of gastrointestinal tract and digestion. Long before the human microbiome revolution occurred, the central attention to Panchakarma (five purifications) was primarily on the digestive tract. The demonstration of the relief in bronchial asthma accompanied by an increase in FEV1 by a Panchakarma—Vamana (medically supervised emesis) was a unique contribution (Dahanukar and Thatte, 1997). Clinically, antiinflammatory plants of Ayurveda like *Boswellia serrata* have shown clinical relief in Crohn's disease (Gerhardt et al., 2001). Chronic smoldering inflammation of the gastrointestinal tract is emerging as a common substratum for diabesity, metabolic syndrome, rheumatoid arthritis, and cancer (Vaidya et al., 2008). RP of

FIGURE 4.3 *Mucuna pruriens* legumes, seeds, and trichomes (under Scanning Electron Microscope).

Panchakarma and Ayurvedic antiinflammatory drugs could open up a novel field for a mechanistic understanding of the aforesaid clinical conditions.

UNIQUE DIMENSIONS OF AYURVEDA THERAPEUTICS

Nanaushadhibhutam Jagati Kinchitdravyam Upalabhyate I
Tam Tam Yuktam Artham Cha Tam Tam Abhipretya II

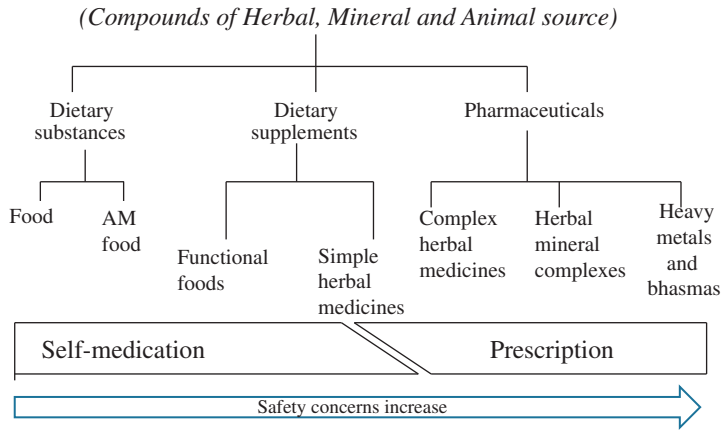
(Charak Su. 26-12)

"There is no substance in the world which cannot serve as medicine through intelligent application. Ayurveda has always cherished such a broad vision for resourcing medicinal substances for its therapeutics" (Joshi, 2003).

Ayurvedic therapeutics (*chikitsa*) is generally classified as *daivya-vyapashraya* (divine therapies: inclusive of wearing precious stones, reciting prayers, chanting mantras, performing homa-havan, etc.), *yuktivyapashraya* (rational therapies: based on logistics of therapeutic principles), and *satvava-jaya* (mindfulness: yoga and meditation). However, the current mainstream Ayurveda practice primarily adheres to the tenets of *yuktivyapashraya chikitsa*. The therapeutic principles for management logistically involve the reversal of pathogenesis that takes into account *nidana parivarjana* (avoiding causative and precipitating factors), *sanshamana chikitsa* (restoration of

TABLE 4.5 Classical Management Approach in Ayurveda

Aahara (diet regimen)	*Hitahara* (healthy diet)
	Mitahara (moderate diet)
Vihara (self-efficacy)	*Achara* (behavioral and lifestyle management)
	Vichara (psychological construct)
	Vyayama (physical exercise)
Aushadhi (medical)	*Bheshaja* (pharmacotherapy)
	Yantra-shastra (physical/surgical therapy)



Plants form important ingredient across the ayurvedic drugs spectrum.
AM food: Ayurvedically modified food, e.g., milk processed with turmeric

FIGURE 4.4 Spectrum of ayurvedic drugs.

physiological homeostasis), *sanshodhana chikitsa* (detoxification procedures for harmonizing human system) and *rasayana chikitsa* (rejuvenative and reparative medicine). The actual clinical management implementation includes *ahar* (diet regimen), *vihar* (self-efficacy), and aushadhi (medical management). Table 4.5 depicts the subclasses of Ayurveda's classical management approach (Raut and Gundeti, 2014).

Pharmacotherapeutics in Ayurveda covers a broad spectrum of drugs: simple kitchen remedies, complex herbo-mineral formulations, preparations of metals, and animal products (Fig. 4.4). However, for this chapter we shall restrict our discussion to herb-based medicines. In Ayurveda, the plant kingdom has been explored for medicinal uses in great depth since ancient times. In the adoptive age, medicines were used in their available natural form; in the cultural period, there was an adaptive age with the art of pharmacy

making modifications in the NPs. Thereafter was the creative age, with the analysis and synthesis of the active plant constituents (Parikh, 1992). During this evolutionary progression of Ayurveda, diverse concepts and subbranches were established. The concept of *guna* essentially constitutes the physical/chemical properties of the material such as density, viscosity, dispersibility, flow properties, etc. (Vaidya, 1992). The concepts of *rasa* (taste), *veerya* (anabolic/catabolic properties), *vipak* (metabolite taste), and *prabhav* (unique efficiency) cover the biodynamic effects of medicinal plant/product. The subbranch of *aushadhi-nirman* and *bhaishajya-kalpana* deals with manufacturing and pharmaceutical developments, whereas the branch of *dravya-guna-karma* deals with Ayurvedic pharmacology.

Much importance is attributed to the habitat, season, and time of collection of medicinal plant material. These factors would influence the concentrations of the phytoconstituents and the methods of collection, preservation, and manufacturing would influence the quality besides the activity of the product (Sharma and Dash, 1985). The aphorism *sanskaro hi gunantaradhanam uchyate* indicates the significance of diverse processes during manufacturing which influences enhancing/modulating of activity and reducing/subduing of toxicity. *Piper longum* is one of the most commonly used ingredients in Ayurvedic formulation. *Chausashti pimpali* is a unique preparation where seed powder of the plant is triturated in its own decoction for specific number of times and duration. This unique preparation is indicated for children for their recurrent respiratory ailments. It has been demonstrated that such a classical method of processing affects the *piperine* content of the product (Raut, 1992).

Besides the aforementioned factors, the product-related attributes such as *ayurvedeeya kalpa* (dosage form), *aushadhi prayoga* (dosage regimen), *aushadhi kala* (dosage schedule), *anupana* (vehicle for administration), and *ahar-vihar* (concomitant diet and lifestyle regimen) are considered as significant determinants for the therapeutic outcome. Guggulu formulations are very popular amongst Ayurvedic practitioners and used in diverse dosage forms as well as in different combinations with other plants for pertinent clinical conditions (Apte, 1988). *Vardhamanprayoga* is one of the distinctive methods recommended where the drug is initiated with a minimal dose and then gets gradually increased to the maximum tolerable dose; it is then subsequently reduced gradually in a reverse order to the minimum the dose it started with. Such cycles of dosage regimen are repeated a number of times depending on the indications and the formulation used. Different dosage schedules and dose administrations recommended in Ayurveda appear to address the facilitation of absorption, drug-food interactions, and biorhythms. Diverse vehicles are identified along with the drug administration for specific indication. To illustrate: the Bhallatak formulation, when studied for its long-term safety profile, demonstrated no mortality in the group of animals that received milk as a vehicle, whereas other groups had severe toxicity (Dineshkumar and Shashikeran, 2008).

A profound aphorism from Ayurveda states *chikitsa nasti shuddhastu yo anyamanyam udiriyet*, which means the therapy that gives rise to another disease is not a pure and proper one.

ORGANIZATION FOR ACADEMIC DEVELOPMENT OF REVERSE PHARMACOLOGY

RP has emerged as a transdiscipline for more productive drug discovery and cost-effective development. The term underlines two important points: it adheres to the core principles of pharmacology and it provides a different perspective in its approach to drug discovery. This different bedside perspective values a human-centric approach over a techno-centric one. It also proposes therapy-centric attention over pharmaco-centric (Raut et al., 2012). The lack of an organized RP approach has delayed by decades the development of new drugs from existing therapeutic experience. Structural and functional organization, multisystem, and multidisciplinary faculty, and an appropriately placed academic location are essential for the growth and development of the new transdiscipline, RP (Raut and Vaidya, 2011).

The organization of RP will have to maintain dynamism, flexibility, and a progressive approach to adopt and assimilate relevant scientific and technological advancements along with the due cognition of a rich, untapped heritage. The personnel involved would have to have state-of-the art skills and knowledge, but it is more important to have an appropriate attitude. Clinicians would necessarily have to evolve as adepts-physician-scientists/vaidya-scientists (Vaidya, 2010b), and the basic scientists, working in laboratories, would have to be more aware of the clinical relevance and applications of their R&D at patients' bedsides. Optimum product standardization and ethical approvals are the prerequisites for RP studies. The expertise and infrastructure needed are mandatory in RP organization. Other valuable infrastructural setups in RP are multisystem research-based clinics, clinical laboratories with specialty research laboratories, human pharmacology units, Ayur- and pharmaco-informatics, documentation and administrative units. Project-specific and need-based consultations and collaborations with specialists of diverse domains have to be explored and encouraged strongly. Table 4.6 depicts structural and functional elements for the organization of RP.

The path of RP is now internationally pursued (Aggarwal et al., 2011), explored (Willcox et al., 2011), and acknowledged (Raut and Chorghade, 2014). Experiential, exploratory, and experimental domains of RP may have academic locations at different national/international institutions. A robust coordination of team and networking by strong leaders are necessary across institutions. The academic location may even be placed in an individual clinic/laboratory/community setup, provided the individual has internalized and grasped the RP organization and has constant linkages and networking with the advanced center of RP. A larger impact of RP and its long-term

TABLE 4.6 Structural and Functional Elements for Organization of Reverse Pharmacology

Structural Organization	Functional Organization
State of the art integrative research clinics	Effective networking and collaborations
Research laboratories for exploratory studies	Liaison with regulatory agencies
Pharmacy unit for product standardization	Intersystem ethics committee approvals
Human pharmacology unit for dynamic/kinetic studies	Integrative research advisory committee/review board
Animal house essentially for safety pharmacology	Regular interactive scientific sessions for debate & discussions
Major hospital and pharma sector for large scale experiments	Dialogue and interviews of traditional healers
Documentation cell with health care informatics	Visits and excursions to biodiversity spots

sustenance demand well-structured and diligently organized training modules. The Medical Research Center of Kasturba Health Society, which is also an ICMR's Advanced Center for RP had organized a two-week ICMR workshop for training in RP from March 26 to April 9, 2011.

Cognizing of the multisystem-multidisciplinary and integrative nature of the RP training program, and appreciation of the importance and significance of such a program for traditional medical research, inspired Vice Chancellor of Maharashtra University of Health Sciences (MUHS), Dr. Arun Jamkar, to announce a Fellowship Program in RP and Drug Development (FRPDD) under the aegis of MUHS. Two of the authors, AAR and ABDV, spearheaded the preparation of the syllabus. A one-year curriculum was prepared by inviting the inputs from individuals/institutions actively engaged in Ayurveda, natural product-research, and drug development. The curriculum proposed has been approved by board of studies of MUHS. The training module gives more importance to active learning over passive training and is intended to develop appropriate attitude, skills, and knowledge in RP and drug discovery. It is expected that these trainees would eventually devise innovations in healthcare research and in methodology of translational research at the interface of Ayurveda and the basic sciences. The early training of the faculty is already in progress. However, having a suitable and eligible multisystem and multidisciplinary faculty is a challenge. In the absence of standard reference book, an anthology of RP publications is in process and an outline of a textbook on RP is ready.

Finally, regular and periodical training through workshops/postdoctoral fellowship programs in RP and eventual transformation and implementations of RP principles and methodologies by trained participants would determine the futuristic spread, growth and development of RP.

CHALLENGES AND OPPORTUNITIES IN REVERSE PHARMACOLOGY

The fact is that the transdiscipline of RP has grown in the milieu of Ayurveda and that too in India; Ayurveda as an organized system of healthcare is neither globally known nor understood. As a consequence, the multinational companies, wedded to the reductionist paradigm of drug discovery, find it difficult to comprehend how RP applied to Ayurveda can be a productive path for new drugs. Ayurveda is not merely an ancient system of health irrelevant to modernity. Ayurveda is still used by 70 percent of 1.25 billion Indians—a vast potential field for novel biodynamic effects. The fundamental principles and practices of Ayurveda are so profound that even its cursory study would convince any open-minded person to mine its wisdom. However, there is no doubt about the stupendous advances that have transformed allopathy into modern medicine. As a result, modern medicine has developed the hubris due to its high-tech success. The humility that allowed Edward Jenner to listen to a milkmaid who said that cowpox prevents smallpox is rare to find (Riedel, 2005). Much hubris prevents a study of the Sanskrit texts of Ayurveda. There is a selective amnesia that most modern drugs owe their success to their origin from NPs. But humility, amidst unprecedented technical progress, is rarely acquired. Dobree puts it well: "It is difficult to be humble. Even if you aim at humility, there is no guarantee that when you have attained the state you will not be proud of the fact." The challenge can be addressed, partly, by some examples of successful products developed through RP for unmet medical needs. The recent Nobel Prize in Medicine received by Professor Tu Youyou is an apt example that illustrates the need for attention to the approach of RP, notwithstanding the long delay from usage in TCM to a global drug (The 2015 Nobel Prize in Physiology or Medicine - Press Release). However, the Nobel committee has stated clearly that the prize is not the recognition for TCM. This is a bit unfortunate because a long usage of a plant in TCM/Ayurveda with clinically proven efficacy and safety is also a major contribution in therapeutics. We observed antimalarial and disease modifying activity with *Nyctanthes arbotristis* at an Ayurvedic hospital with state-of-the art response markers (Godse, 2004). It is suggested that such an interface research in RP would not lead to opportunity loss or long delays in discovering globally relevant natural drugs from ancient systems of medicine.

The irony is that the traditional eminence-based mindset in Ayurveda is late in adopting biomedical advances and technology that are congruent with

its basic principles but are badly needed for patient care. This wariness and phobia of advanced technologies also carries over to the relevant science and techniques used by RP. It appears alien to the Ayurvedic faculty with an eminence-based mindset. Among them, there is an element of ancestral vainglory and complacency that prevents them from grasping the opportunities RP offers for evidence-based Ayurveda. This is an even greater challenge than the ones posed by modern medicine. This is primarily so because the experiential robust documentation of hits, at the bedside, is within their domain. In India, a novel initiative has been taken to train and develop vaidya-scientists (Patwardhan et al., 2011). The returns are remarkable, as vaidya-scientists are competent to engage in RP and scientific research with a high motivation.

Another major challenge for RP is its need of a transsystem clinical and basic infrastructure, with a state-of-the art capability to develop a new drug from a clinical hit. Currently, there is a separate Ministry of AYUSH established by the government of India. It is hoped that with this empowerment the public perception of AYUSH will hopefully change, by a judicious adoption of science and technology compatible with fundamentals of Ayurveda (Patwardhan, 2015). There are hardly any integrated medicine departments in India. There is more hope for RP from the universities abroad where many medical colleges do have Departments of Complementary and Alternative Medicine. It is desirable that these departments, in collaboration with their clinical pharmacology units, develop training and research in RP. As most of these colleges do not allow Ayurveda, their chances of getting clinical hits and leads are meager. But there is a vast potential of paraclinical studies for the leads and candidate NPs already discovered in India. Table 4.7 lists some such plants and the relevant experiments for correlates.

The biggest challenge facing a serious enterprise in RP is the mindless marketing of the Ayurvedic and Chinese medicines as over-the-counter DS in the West. These are marketed without any regulatory approvals and with all sorts of claims for health. As a consequence, when scientific evidence generated by RP is presented, the earlier noise in the marketplace affects the credibility of new data. In India, we have taken care of the proper categorization of natural drugs into three groups with their respective standards and regulatory guidelines: (1) Ayurvedic, Siddha, and Unani drugs under AYUSH; (2) phytopharmaceuticals under the drug controllers; and (3) food supplements (ayurceuticals) under Food Safety and Standards Authority of India (FSSAI). This clarity offers unprecedented opportunities to discover and develop, through RP, a remedy under a specific category.

FUTURE DIRECTION AND SCOPE OF DIFFERENTIATION IN REVERSE PHARMACOLOGY

RP, being a very new paradigm, has to face a degree of uncertainty as to its future direction. The analogy of RP to clinical pharmacology would serve as

TABLE 4.7 Opportunities for Reverse Pharmacology Correlates (Vaidya, A.D.B., 2006; 2010b)

Ayurvedic Drug	Medicinal Plant	Demonstrated Action	Para-Clinical Studies
Arogyawardhani	*Picrorhiza kurroa*	Hydro-choleretic	Gallstone prevention
Kapikachhupak	*Mucuna pruriens*	Antiparkinson	Neuroplasticity
Amrutbhallatak	*Semicarpus anacardium*	Antiarthritic	Chondrocyte stem cells
Asthisandhanak	*Cissus quadrangularis*	Fracture-healing	Hydroxyapatite laying
Chashashth pippali	*Piper longum*	Antiasthmatic	LTB-4 antagonist
Ashokarishta	*Saraca asoca*	Antimenorrhagic	Vascular stability
Ashwagandharishta	*Withania somnifera*	Anticancer	Immune surveillance
Rasavanti	*Berberis aristata*	Antiglaucoma	Less intraocular pressure

signpost for an appropriate direction to this new transdiscipline. Clinical pharmacology actually evolved as human pharmacology for studying effects of drugs on human body and what the human organism does to the drugs. Unfortunately, rather than growing as a discovery transdiscipline which would enrich human biology, clinical pharmacology was grabbed and dwarfed by the drug industry. As a consequence, it is sad that it got restricted to Phase I to Phase IV trials. The vast potential of clinical pharmacology was thwarted. For the future direction of RP we have to be cautious that the transdiscipline would be adopted, expanded, and made fruitful by an active collaboration of drug discovery scientists from academia and industry.

The very transdisciplinary nature of RP necessitates its development as an academic endeavor. The initiation of RP being at the bedside, excellent and state-of-the art clinical facilities is at its core of development. The inspirational roots of RP also lie in robust traditions of Ayurveda, TCM, etc.; hence, it is desirable that teaching hospitals of these systems provide a base for observational therapeutics and experiential studies (Vaidya, 2010a; Vaidya and Raut, 2006). Bridges will have to be built with super-specialty clinical research units for relevant human exploratory studies. These units should have linkage with experimental/cellular/biochemical pharmacology for pursuing the clinical hits and leads in appropriate in vitro and in vivo models. Besides the stress on novel clinical pharmacodynamic data providing hits and leads, RP can open up new domains in human biology. Such new

generalization at human level could lead to an impact on the cumulative reductionist data from life sciences.

RP has a vast scope for differentiation and emergence of unique specialties. It cannot be overemphasized that clinical and therapeutic freedom and pluralism would provide a rich field for fertile hits and leads of novel biodynamic effects. However, the current undue stress on evidence-based medical practice has often limited the chance of serendipitous discoveries and their follow up. Ayurvedic epistemology has a clarity and simplicity that permits pluralistic therapeutic approaches. The latter are primarily concerned with the reversal of pathogenesis in an individual patient. The training and development of vaidya-scientists, as a specialty, was intended to equip the faculty with a strong foundation in *shastra* and a deep acquaintance in life sciences (Vaidya, 2010b). This would be the front-line differentiated specialty needed in RP. This group would generate hits in experiential stage of RP. For in-depth exploratory studies, objective variables of clinical/laboratory markers are most vital. Noninvasive clinical methods, current biochemical/immune/microbial/molecular markers, and imaging/scopy techniques are essential for the documentation (Hastak et al., 1997; Joshi et al., 2004, 2011; Sheth et al., 2006; Godse et al., 2011). In the future, laboratory medical scientists who focus on RP studies would emerge as investigative reverse pharmacologists. The paraclinical in vitro and in vivo studies in RP demand a unique orientation to novel clinical drug phenomena. For the mechanistic understanding of the drug actions, relevance of pharmacokinetics/metabolism and relevant safety profiles, RP would require experts with a foundation in basic sciences. The specialties which would emerge from the differentiation are reverse pharmacodynamists, reverse pharmacokineticists, safety pharmacologists, and cellular/molecular pharmacologists for NPs. Such a wide scope of super specialties in RP may appear daunting at present. But the vast number of clinically documented hits and leads demand the rigor and expertise to mine the field effectively. The current unmet healthcare needs in communicable and noncommunicable diseases as well as the emergent new challenges could be substantially met with by such a transdisciplinary differentiation of RP.

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