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# Conceptions of Potency, Purity, and Synergy-by-Design: Toward Developing a Sowa Rigpa Medical Theory-based Approach to Pharmaceutical Research

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# Conceptions of Potency, Purity, and Synergy-by-Design: Toward Developing a Sowa Rigpa Medical Theory-based Approach to Pharmaceutical Research

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Sowa Rigpa institutions and practitioners have growing interest in examining and legitimizing Sowa Rigpa formulas vis-à-vis pharmacological research methods, seeking scientific validation of what they view as 'potency' and 'purity' for their formulas. Likewise, pharmacology researchers have demonstrated renewed interest in herbal medical traditions in mining for new drugs to address resistance, toxicity, and optimize what they view as 'potency' and 'purity.' However, differing conceptualizations emerge when the pharmacological drug discovery process is examined to determine what is being analyzed, how it is doing so, and what assumptions underlie such methods. Whether a formula is 'active,' 'toxic,' or 'effective' hinges on assumptions, processes, and methods that typically have low fidelity to how Sowa Rigpa formulations function from the Tibetan tradition's perspective and are actually administered to patients. This paper argues that standard mainstream biochemical pharmacology screening methods may not be suitable for analyzing Sowa Rigpa formulas, as they are traditionally compounded and understood to function in concert with multiple physiological pathways, rather than one specific target. As such, we examine the pharmaceutical research processes to identify points of adherence and divergence with conceptions of 'potency' and 'purity' in Tibetan medical theory. We believe pharmacological research institutions will be receptive to traditional Sowa Rigpa *menjor* (*sman sbyor*), or 'medicine compounding,' theory due to benefits it could provide biomedical drug discovery via complementary understandings of compound synergy and distinctly different concepts of toxicity and purity. Accordingly, we suggest that efficacy, activity, and safety of Tibetan medicinal formulas will be more accurately assessed by retaining fidelity to its own conceptions of potency and purity.

**Keywords:** Sowa Rigpa, pharmacology, synergy, integrative medicine, toxicology.

## Introduction

Textually formalized in the twelfth century, Sowa Rigpa (*gso ba rig pa*), or the 'science of healing,' is a scholarly Asian medical system also known as Tibetan medicine. It is still practiced as the dominant health care system in many regions of the world, particularly in Tibet and Tibetan-populated parts of China, Himalayan communities, areas of Tibetan refugee settlements in India, Nepal, Bhutan, Mongolia, Buryatia, Russia, North America, and Europe (Craig and Adams 2008; Yeshi et al. 2018). The canonical root text called the *Four Tantras* (*rgyud bzhi*), written in classical Tibetan, is still memorized by students and used extensively by physicians,[1] along with hundreds of commentaries composed over centuries that provide authority for theory and practice.[2] Sowa Rigpa shares extensive histories, texts and practices with Indian Ayurveda, Chinese medicine, and Greco-Arabic traditions, such as Unani. Various regions have developed Sowa Rigpa traditions specific to their geographic and socio-ecological context.[3] *Menjor* (*sman sbyor*), or 'medicine conjoining,' is the medicine compounding theory and practice within Sowa Rigpa.[4] It is a subdiscipline of *dzéjor rikpa* (*rdzas sbyor rig pa*), or the knowledge of how substances combine, in which the subdiscipline addresses making substances with therapeutic effect. *Menjor* provides a corollary in our comparison with biomedical pharmacology, but with important and distinct concepts and terms specific to each intellectual history and tradition. In this paper, we acknowledge that one epistemology can never fully map onto another. However, correlating epistemologies and understanding basic structuring of concepts is foundational for collaboration between two intellectual traditions. This paper focuses on the Tibetan tradition of Sowa Rigpa (*bod lugs gso ba rig pa*) and its *menjor* specifically, but content may apply to other Sowa Rigpa traditions as well.

Sowa Rigpa institutions and physicians have growing interest in seeking compound evaluation by biomedical pharmaceutical and clinical research methods (Reuter et al. 2013; Luo et al. 2015) due to pressures to provide evidence for efficacy and safety in national and international domains (Saxer 2013; Wangchuk & Tashi 2016). Additionally, increased production of Sowa Rigpa formulas, for both domestic and global markets, has required development of certain standards for production (Saxer 2012). Yet, this widening industrial Sowa Rigpa pharmaceutical assemblage (Kloos 2017) raises concerns within the Tibetan medical field of proper formula efficacy as it threatens to untether treatment and medicine production from physician care. National and international safety and efficacy evaluations of Sowa Rigpa formulas rely on biochemical analytics in collaboration with pharmacology laboratories in both academia and industry. Each collaborative side often works from different meanings of 'purity,' 'toxicity,' and 'efficacy.' Standard biomedical analytic processes are not designed to address distinct Tibetan medical conceptualizations of 'potency,' 'activity,' and multi-compound synergy-by-design. In Sowa Rigpa research, few social scientists and Sowa Rigpa physicians are familiar with biomedical drug discovery and pharmaceutical research processes. As such, they may mistakenly read results of pharmacological analysis of Sowa Rigpa medicines as if the analytic methods used actually assess activity and toxicity of whole formulas as they behave in patient bodies (see Schwabl and van der Valk 2019). Such limitations are recognized by experienced scientists who analyze flaws in published studies to design new ones (Brown et al. 2018). Likewise, it is also important for Sowa Rigpa practitioners to consider if the analytic methods applied are appropriate for the formula being tested. National and international restrictions on Tibetan formulations do not consider how initial toxic ingredients may be chemically transformed into therapeutic forms through Tibetan compounding (Craig 2011a, 2011b, 2012), such as liquid mercury converted to a mercury sulfide form thought non-toxic. Improved research approaches to assess activity or toxicity of compounds in the form actually administered to patients may help remove obstacles for Sowa Rigpa globally (Schrempf 2015). This article discusses limiting assumptions of historical approaches and highlights recent advances that contribute further rigor to future collaborative endeavors.

Some biomedical researchers have growing interests in collaborating with traditional medicine specialists toward new drugs and combination therapies for unmet medical needs (e.g., acquired drug-resistance and off-target side-effects). This is evidenced by the Nobel prize awarded for discovery of artemisinin (*qinghaosu* '青蒿素' in Chinese) to treat malaria (Shen 2015) and antimalarial Sowa Rigpa drug leads (Wangchuk et al. 2012, 2013). However, many of these studies are focused on discovery of new, patentable, single agents and do not address formulas as they are traditionally used. 'Magic bullet' monotherapy formulas have been used extensively in biomedicine for the past century (Strebhardt and Ullrich 2008), but they often produce side-effects and select for resistance over time (Zhang 2005). This approach has driven pharmaceutical research on a constant search for new compounds to treat the next drug-resistant disease agent. Decades of observing how resistance emerges due to these modern monotherapy

treatments has shifted pharmacology to embrace the concept of 'combination therapy,' where properly designed positive combinations of two or more drugs can increase efficacy (Butler 2019) and/or decrease toxicity for the mixture relative to individual components (Jia et al. 2009). Unfortunately, biomedical analysis of drug combinations is complex and requires more elaborate and differentiated testing than commonly done in most drug screening labs (Foucquier and Guedj 2015). We propose that better understanding of principles behind standard medicinal formula testing may enhance Sowa Rigpa practitioners' ability to assist pharmacological experiment design with direct relevance to traditional medical practices.

Different types of collaboration analyzing traditional medicines have developed over the last decade. One type occurs where a traditional medical institute provides single plant materials or other multicompound formula parts for chemical analysis. The samples undergo typical 'natural product' screening operations, where the whole plant or formula moves through staged chemical extraction to separate potentially 'active' ingredient(s) before testing in a specific disease model assay.[5] A second collaboration type seeks to evaluate an entire formulation to validate and define specific biological activities affected, such as recent network pharmacology studies of the Tibetan medicine Drébu Sum Tang (*'bras bu gsum thang*), discussed later.[6] In a third type of collaboration a Tibetan medical institute gives a pharmacology lab a medicinal substance, or formulas containing the substance, to test for safety and composition. An example here is *tsotel* (*btso thal*, lit. 'refined ash'), an organometallic mercury sulfide complex used in several important Tibetan medicines including precious pills. Considered the pinnacle of Tibetan *menjor* accomplishments, *tsotel* is often referred to as 'purified' mercury, a quintessential exemplification of the detoxification/potentiation approach through multicompound formulas in Sowa Rigpa (see Gerke 2013a, 2013b, forthcoming). However, due to the known toxicity of the initial ingredient, elemental mercury, it has raised concern internationally and demands demonstration of its safety mechanism through scientific investigation (Sallon et al. 2006, 2017; Liu et al. 2018).

In this paper, we present key paradigms underlying both Tibetan *menjor* and pharmacology in their distinct concepts of potency, toxicity, and activity due to multicompound synergistic effects. We discuss main Tibetan *menjor* theoretical frameworks for synergy and detoxification that prioritize multicompound formulas over single compounds. For pharmacology, we describe major historic developments in drug discovery methods, highlighting both strengths and limiting assumptions. We discuss collaborative contexts within which pharmacological analysis of Sowa Rigpa substances occur. We also describe the basic drug discovery process often applied for Tibetan formula analysis by introducing basic steps of modern drug discovery highlighting noteworthy cases of antibiotic, anticancer, and antiviral drug discovery. For complex medicinal substances, such as Drébu Sum Tang and *tsotel*, we present the limitations of standard chemical analytical techniques and discuss recent advances.

A supplementary table summarizing comparative terms and concepts of pharmacology and *menjor* discussed within is available at (See Supplemental Table 1. Key Comparative Concepts in Pharmacology and Tibetan Menjor (online only)).

## Distinct Concepts of 'Purity': Basic Units Begin the Conversation

Pharmacologists employ the concept of 'purity' differently than the equivalent concept *jangpa* (*sbyang pa*) for Tibetan *menjor*. In pharmacology, 'purity' derives from the idea that a pure substance contains only one type of atom or molecule (Pauli et al. 2014). In *menjor*, purity is the degree to which a substance does no harm, often requiring the combining and fusing of several complex substances (*rdzas*) to achieve a 'pure' (*dag pa*) substance. Pharmacological collaborations that engage concepts of 'purity' and 'toxicity' require a foundation of basic units upon which chemists and Tibetan physician-pharmacists can work together from their respective paradigms. For example, the word 'atom,' often synonymous with 'element,' designates the smallest unit of a particular type of physical matter. While derived from the Greek *a tomos,* meaning 'not cuttable,' it is recognized in modern pharmacology that atomic matter is primarily energy—$E=mc^2$ (Rainville et al. 2005). Accordingly, it is the energy form, or orbital space of atoms that gives elements their specific chemical properties—not a solid structure. Sowa Rigpa *menjor* also developed out of early descriptions of matter and energy. Early Buddhists in India described minute units of matter with subtypes that corresponded to the four basic elemental forms. Later, the concept developed to that which is characterized in Tibet[7] as dültren (*rdul phran*), or momentary and infinitesimally small partless particles. *Dültren* are understood to exhibit properties dependent on context, rather than as inherent properties of the particles themselves. Thus, the concept of 'purity' in *menjor* is not linked to a single-type particle and relates closer to activities of the elemental dynamics.

The elemental dynamics are five interactive properties known in Tibetan as *jungwa nga* (*'byung ba lnga*), often translated as 'elements.' Herein we use the term 'dynamics' to emphasize their understanding as properties exhibited by matter and energy, not the physical substances. The five dynamics are referred to simply as 'earth,' 'water,' 'fire,' 'wind,' and 'space,' defined by their respective properties of solidity/stability, cohesion/fluidity, maturation/heat, motility/movement, and interactive space. For example, the physical substance of water has all five dynamics of solidity, cohesion, heat, and motility. *Materia medica* in Tibetan medicine, as with all matter including our bodies, are classified and understood according to properties of these dynamics.[8] Here, we suggest that 'elemental' dynamic properties in Tibetan *menjor* provide a working concept analogous to the pharmacological sense of 'chemical properties'. A chemical property is any property that becomes evident from observing a substance's dynamic behavior. Chemical properties are emergent, just as in Tibetan *menjor*, elemental 'dynamics' describe the emergent nature of interactions, the mode of reaction between substances, not the substances themselves. Pharmacologists characterize atomic elements as behaving in different ways depending on temperature, pressure, context, and relation to other reactive substances. As such, atomic elements could demonstrate behaviors of each of the five dynamics depending on context. For example, water molecules (containing elements of hydrogen and oxygen) can demonstrate qualities of cohesion and solidity in both solid and liquid forms—which are characteristics of the water and earth dynamics, respectively. However, in gaseous and superfluid forms where water demonstrates no viscosity, water manifests the highly motile qualities of the wind dynamic and the heat-producing kinetic energy of the fire dynamic. In interaction with other substances, water molecules may provide a structural substrate for other molecule species to interact, in which its behavior would demonstrate the space dynamic.

## Understanding Functional Activities in Sowa Rigpa Menjor

Sowa Rigpa physician-pharmacologists have systematized *materia medica* (see Figure 1) according to a substance's proclivity to manifest interactive properties of combinatorial dynamics in what they call 'taste' (*ro*), 'potency' (*nus pa*), 'post-digestive taste' (*zhu rjes*), and 'quality' (*yon tan*). They assess how compounds interact physiologically in both functional and dysfunctional pathways, defining the nature of properties according to classes through observations and techniques not unlike pharmacological methods of an assay. The highly systematized techniques determining qualities and functional activities of *materia medica* in Tibetan *menjor* known as 'recognizing signs' (*ngos 'dzin rtags*), or 'markers' of pathway activities, are similar to techniques used for its patient diagnostics (Tidwell 2017). Thus, both biomedical and Sowa Rigpa intellectual traditions have developed distinct systems of investigative (analytic/direct perceptual) techniques for qualitatively and quantitatively assessing and measuring the condition of a patient or presence, amount, or functional activity of a medicinal substance. A comparative analysis of different assumptions and techniques pertinent to each tradition's assay approach is beyond the scope of this paper. However, we suggest mutual recognition of each approach as a bridge toward collaborative research aims.

In Sowa Rigpa, 'taste' is a cascade of activities that commence with the initial interaction of a substance with the tongue and related taste faculties in the mouth. Neuroscientists are beginning to understand such a concept as they assess how the sense of taste results from specific chemical types interacting with spatial arrays of particular sensing molecules, or 'taste receptors' (Adler et al. 2000) in the mouth, nasal, and gut passages (Margolskee

**Figure 1. Classical Tibetan medical thangka scroll painting reproduction based on Lhasa Men-Tsee-Khang set, initially commissioned in 17th century and re-developed in the early 20th century. The scroll painting depicts several geo- and woody/herbaceous medicinals in Sowa Rigpa materia medica. Scroll paintings created by Dharmapala Thangka Centre, School of Thangka Painting, Kathmandu - Nepal <www.thangka.de>.**

2015). Though seemingly simple, human 'taste' is actually a very complex process of chemical identification for survival of an organism (Shallenberger 1997; Margolskee 2015). In Sowa Rigpa, initial taste complexes facilitate digestive processes in the gut that react with the ingested substance to transform and transmit metabolites into pathways and trajectories across organ systems, body fluids, bodily constituents, and mental processes. Taste describes the physiological activity that specific chemical types initiate (Shallenberger 1997). 'Post-digestive taste' characterizes the activity that occurs once the substance reacts in three gateway reactors in the gut that modify the activity sequentially—'decomposing *béken*,' 'digesting *tripa*,' and then 'essence-waste separating heat-associated *rlung*.'[9] Post-digestive taste can be intentionally cultivated in a substance through heating, drying, and processing techniques that manipulate the elements. 'Potency' is the final activity of the substance, resultant from its taste profile reacting in the gut. These three characteristics—taste, potency, and post-digestive taste—differentially describe a substance's overall activity by its body pathway effects, delineated further according to 'qualities' that determine activities on physiologic function and disease.

Some substances function as predicted by their taste, some by their post-digestive taste. Others contradict the predicted effect from taste and/or post-digestive taste but act according to their potency. Substance characteristics are classified, enumerated, and explicated to provide the foundation upon which a formula is calculated and composed. For example, a substance is primarily classified according to its overall warming or cooling activities due to the dominant dynamic combination driving its physiologic activity. It is then elaborated according to its qualities classified by taste.[10]

## Material Processing & Formula Compounding: Toxicity, Purity, and Efficacy

The *Four Tantras* detail many minerals, gems, precious substances, flora, and fauna used in the *materia medica* which have toxins *duk* (*dug*) that are harmful to the body, and at times lethal, if not properly detoxified—including mercury (Yuthog Yönten Gönpo: 75-89; 626-630) (see Figure 2, depicting Sowa Rigpa perspective on origin of toxins (e.g., ibid 2008: 589-590)). Thus, Sowa Rigpa practitioners have developed extensive systems of transforming substances to remove and transmute toxicity and optimize therapeutic value—allowing medicinal qualities to emerge, so to speak. This is referred to as *düljong*, 'taming and purifying,' in which medicinal substances are prepared through distinct techniques to develop the medicinal qualities of a substance, detoxify (*dug 'don pa*), 'tame' by eliminating harmful components, and 'purify' it by retaining and imbuing medicinally-potent components and qualities (ibid: 691-700).*Duk* is defined as an entity difficult or 'unsuitable' (*mi rung pa*) to 'metabolize' (*'ju ba*), or to which the body has metabolic 'resistance' (*mi 'phrod pa*, *ma zhu ba*). The *Four Tantras* define 'metabolize' as proper separation of nutritional essence and waste product, and their mobilization to respective bodily constituents and excrements. Improper metabolism, vis-à-vis interference of *duk,* causes bodily harm. Potencies are 'smoothed' (*'jam btsal*) and formulas are developed through processes that heighten certain qualities of a substance's elemental dynamics, creating greater potencies and directed effects. Certain elemental dynamic combinations have 'affinity' (*mthun pa*) that heighten their joint activity, whereas others adversely relate by repelling, destroying, or eliminating activity. 'Affinity' and 'adversity' (*mi mthun pa*) relationships are integrally considered in compounding formulations. Expelling toxins and developing the desired therapeutic effect is referred to as 'purifying' (*sbyong pa; dag pa byed pa*). Taming and purifying involves adhering substances to other components, substrates, catalysts, and transformative fluids to make more stable compound forms.

**Figure 2. Classical Tibetan medical thangka depicting the origin of compounded toxins as described in the *Four Tantras* and *Blue Beryl* commentary, followed by examples of naturally occurring poisonous flora, fauna and substances. Scroll paintings created by Dharmapala Thangka Centre, School of Thangka Painting, Kathmandu - Nepal <www.thangka.de>.**

Synergy is a term used in pharmacology to describe an effect of two or more agents in combination that is greater than their simple additive effect (Roell et al. 2017). Since multiplying effects of combined dynamics, tastes, post-digestive tastes, potencies, qualities, and other processing contributions can heighten formula potency (*phan nus*), the term synergy similarly characterizes such interactions and processing methods. We call the compounding approach based on this theory 'synergy-by-design.' Contemporary Tibetan medical physicians use the term *düljong* as a Sowa Rigpa corollary to the pharmacological processes of developing drugs through compounding principles. This is because *düljong* provides the classic Sowa Rigpa compounding framework for 'taming' toxicities, 'purifying' multiplex compounds, and developing medicinal effect.

In making any Tibetan medicine formula, a physician-pharmacist begins with a base medicinal compound characterized by its taste, potency, and post-digestive taste, as well as overall physiologic effect. Ideally, the physician-pharmacist will have crafted the proper potency profile of the substance through preliminary steps in identification, harvesting and preparation before formulating it with other substances. Though single substances contribute to Sowa Rigpa's extensive drug library, monotherapies are considered susceptible to undesirable effects, and thus formula design focuses on multicompound forms. The following section outlines medicinal specimen preparation and formula development. The section is based on the *Four Tantras* instructions for a medicinal flora class in which the whole plant is used, called *ngo men* (*sngo sman*), which we call 'herbs' here for short.[11] Although specific to this class, the process illuminates all medicinal substance preparations prior to formulations.

## Critical Steps of Medicine Compounding

Herbs are generally recognized as rough and cooling in potency (ibid: 66; 697-698).They tend to have thick mucosal constituents that tend to block *rlung* pathways, causing fire-accompanying *rlung* in the digestive pathway to expel heat externally and desiccate/deteriorate bodily constituents (ibid: 697).Thus, the potency of each herbal ingredient must be prepared properly and 'smoothed' by combining it with other compatible medicinal substances, or by preparing it as a particular concentrated syrup decoction (*khaNDa*) to avoid undesirable effects and focus medicinal activities toward physiological targets (ibid: 698).

Preparing herbs follows a set of practices[12] unique to Sowa Rigpa, though vary regionally in implementation. The practices are methods to cultivate medicinal qualities in harvested plants and when compounding formulas. They begin with harvesting an herb in the proper environment, time, and conditions appropriate to maximizing desired therapeutic qualities. One removes initial 'toxicity,' including mechanical removal of indigestible and metabolically-resistant parts, then dries and stores the specimen according to its potency and qualities (thereby potentiating the specimen).[13] Subsequently, one 'smooths' the specimen by balancing the potencies vis-à-vis processing and formulation with other medicinal substances. This is the key step that drives a multi-formulation approach described in the *Four Tantras* (ibid: 693, 697-700). The three methods for smoothing combine substances to create a balanced formulation that: (1) complements tastes profiles and potency characteristics to address the overall hot-or-cold-nature of the condition to be treated; (2) directs the formula toward a specific target organ, fluid, or pathway imbalance; and (3) minimizes deleterious effects of aggravating *rlung*, extinguishing the digestive fire, and deteriorating bodily constituents. The final step, called 'compounding method for suitability' (*'phrod par sbyar ba*), directs the overall formula toward the appropriate taste, potency, or post-digestive taste through further enhancement.

Other flora, fauna, mineral, and metal substances go through similar processes of proper identification, harvesting, detoxifying, smoothing, and compounding. However, before detoxification, geologic materials often undergo additional steps of specialized rinse-washing and removal of undesired impurities and oxidation products. Detoxifying often requires adding substances to expel toxins, rigorously 'smooth,' cause caustic reactions, and dry, heat, and cook. Furthermore, specific substances are integrated along the way to direct therapeutic effect. Intermediary steps also break down, open, and transform substances through further conjoining. Geomedicinal materials are often enclosed in specialized vessels and adhered to substrates where they are cooked, burned, incinerated, and otherwise modified.

Sowa Rigpa formulation also involves integration with or adherence to a medicinal vehicle or substrate, *menda* (*sman rta*), the medicine chariot or horse, which delivers the proper activity to the patient. *Mendas* may variably be part of 'taming' toxicities, smoothing function or directing formula activity. Formula effectiveness is characterized by the degree to which potency is properly imparted to patient, as formula components are designed to affect specific physiological pathways without toxicity effects, digestive fire debilitation, or bodily constituent harm.

Throughout this process, detoxifying, smoothing, and directing the formula's potency are acts of 'purification' and 'potentiation.'[14] Processing creates different 'efficiencies' in delivering a formula's potency that differ in speed and resultant effect dependent upon degree and type of processing. Textual indications provide the ideal, but physician-pharmacists differentially employ steps dependent on their tradition and resources.

*Tsotel* quintessentially demonstrates the ability for Tibetan medical physicians to tame, purify, and direct the potency of a substance that is normally considered highly toxic. Practitioners describe the process of detoxifying mercury as 'purification,' whereby it is conjoined with sulfur, and numerous herbs, metals, and mineral components requiring many days of processing. The initial compounds used to make *tsotel* include elemental (toxic) mercury and various heavy metals. The very process of making *tsotel* requires primarily ash forms of geologic and organic substances and other *materia medica* ingredient additions, in addition to compounding procedures to detoxify and 'smooth' the final product. Because of its extensive processing, *tsotel* is produced infrequently, rarely by individual physicians, and almost never in other Sowa Rigpa traditions (Yeshi et al. 2018).

It is important to note that this processing is a chemical transformation of mercury, so that it is no longer the same substance. The processing is analogous to that used in modern nanotechnology (Lee et al. 2013). *Tsotel* is used to reduce toxicity and/or heighten potency of various formulas including precious pills, by acting similar to a *menda* or carrier. This also aligns with modern mechanistic use of nanoparticles for drug delivery (Jong and Borm 2008).

By studying stages in making *tsotel* and the final product, a wide spectrum of Sowa Rigpa formulas can be understood since *tsotel* compounding epitomizes many *menjor* production principles (Troru Tsenam 2012).

Tibetan *menjor* has evolved with significant historical developments. Yet, given the text's position of authority within the tradition, the contemporary framework has largely retained fidelity to theory presented in the *Four Tantras*[15] despite regional differences in plants utilized, therapies implemented, and illnesses recognized (Boesi 2006). Conversely, the developmental history of Western pharmacology experienced some key turning points. We detail those related to potency, purity, and synergy-by-design in the following section.

## Fundamental Historical Developments in Pharmacology

Modern pharmacology has historical roots in an early European medical context with characteristics that resemble Sowa Rigpa. The work of Hippocrates (460-360 BC) contributed to a paradigm in which all disease originates from imbalance with nature. Rebalance is achieved through herbal remedies, diet, exercise, and rest to restore alignment of the four humors and relationship between the person's internal nature with the natural state of the external environment. Much of the approach and *materia medica* in the Hippocratic corpus, including both patient care methods and medicinal properties of plants, continue to strongly influence contemporary practitioners of biomedicine over 2000 years later (Hanson 2006).

However, in the late 1800s, German biologist and medicinal chemist Paul Ehrlich affected a paradigm shift in the development of pharmacology. From the patient-focused methods of Hippocrates, Ehrlich developed a concept to design disease-specific chemical compounds he termed 'magic bullets' (*magische Kugel*) aimed toward microscopic biological invaders (Strebhardt and Ullrich 2008). This earned Ehrlich the name of father of modern chemotherapy and drug discovery methods (Bosch 2008). His early research showed that chemical dyes could be used to color specific cellular parts and types, and these new tools allowed scientists to quantify the differences in biological response to different compounds based on changing patterns of staining. He observed microscopic changes in staining correlated with biological changes such as growth and death of cells, and further related observed 'activity' to specific chemical compositions.

Ehrlich's use of staining led to the first systematic cell-based assays using dyes as 'markers' of biological change. He promoted a theory that chemical substances with special affinities for pathogenic organisms could be designed to selectively eliminate such pathogens from the body (Winau et al. 2004). 'Affinity' in the chemical sense refers to the attractive force between substances or particles causing them to enter into and remain in chemical combination. This concept also applies to chemical substances having affinities to organisms. The magic bullet was Ehrlich's term for an ideal therapeutic agent that killed only the organism targeted and was safe for the host. He hypothesized specific structural orientations of chemical features of a compound could render it more toxic to the pathogen than to host organism. In his 1908 Nobel prize acceptance speech, he described the idea of how a drug works based upon a specific chemical size, shape, and charge that complements the biological target receptor—a concept we now call 'pharmacophore' (Yang 2010), and coined the term 'chemotherapy' (Ehrlich 1954).

Ehrlich's most notable drug discovery was a chemical transformation of arsenic (As) that reduced its toxicity in animals and made it useful for treating syphilis. Ehrlich

developed the patented drug known as arsphenamine, or Salvarsan (see Figure 3A), from the poisonous metal arsenic by altering its chemical form and thus toxicity, an approach conceptually similar to 'taming toxicity' in Tibetan *menjor*. Salvarsan became the first patented, highly profitable, global wonder-drug that solidified the single-drug approach for most of that century. However, recent experiments using modern methods suggest the active material is actually a mixture (Lloyd et al. 2005) (Figures 3B and C).

The single-drug approach uses a lock-and-key type model to describe drug activity and specificity. Similar to a key, a drug geometrically fits the appropriate active site (lock) of an enzyme or other biological target (Figure 4A). Given the correct key-like properties, the drug could then lock or unlock a biological function in need of repair. An ideal compound could uniquely destroy a pathogen with specific drugs binding specific biological receptors of the pathogen target. Using the concept of affinity, the ideal medicine—the magic bullet—would have high affinity, vis-à-vis chemical shape and property matching to the receptor. It would also have high 'efficacy,' or a high degree to which it performs the locking or unlocking. 'Potency' in drug discovery is a measurement based on dose-response curves in a given assay.

Ehrlich's pharmacophore methods used cell and animal-based assays to systematically evaluate isolated chemicals. These methods led to discovery of other patentable antibiotic and antiviral 'wonder drugs' that, along with related public health measures, dramatically decreased deaths from infectious diseases during the 20th century. Since that time, biomedical research has primarily focused on

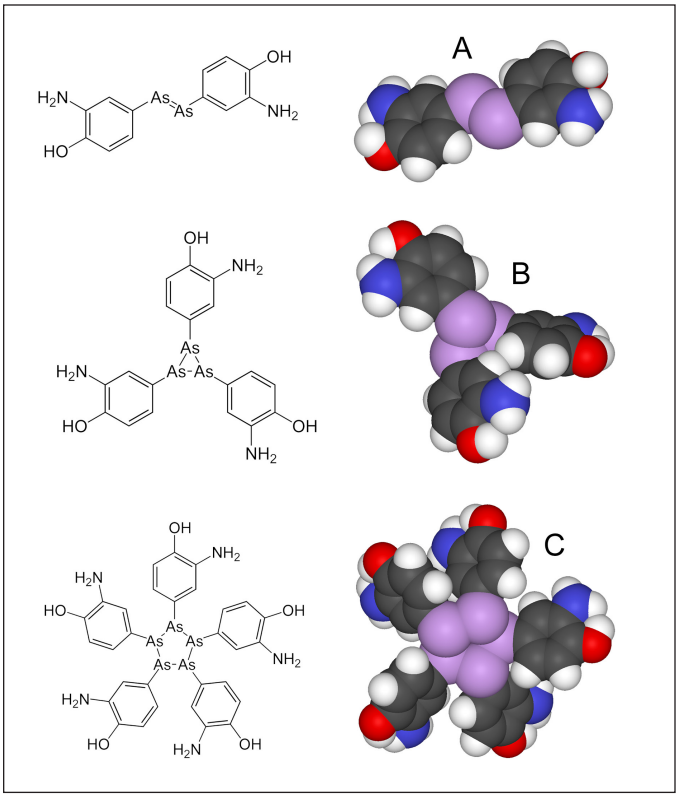


**Figure 3. Salvarsan–the first 'Magic Bullet.' Chemical line and atomic 'space filling' models arsenic-As (purple) complexed with carbon (black), oxygen-O (red), nitrogen-N (blue) and hydrogen-H (white). A) Theoretical single structure containing two arsenic-As atoms original proposed by Ehrlich. B) and C) Recent experiments find 2 cyclic forms with 3 and 5 arsenic-As (purple) dominated the active mixture. It is thought a further oxidized form is actually responsible for the biological effect on the pathogens in vivo, but none of form A) was observed.**

(Salvarsan-montage. Wiki Commons, 2006: <https://commons.wikimedia.org/wiki/File:Salvarsan-montage.png>)

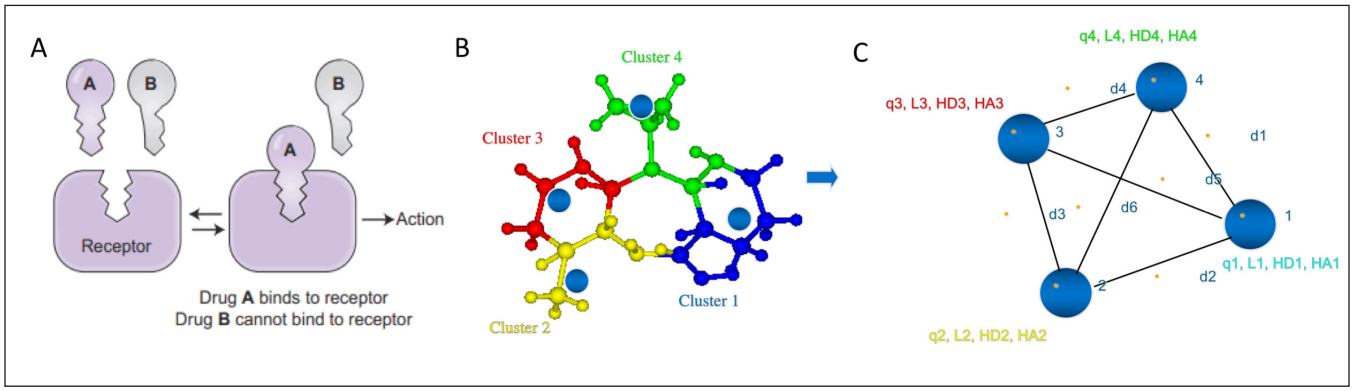


**Figure 4. A) 'Lock and key' pharmacophore models of drug activity. Drug compound (A) with complimentary shape and chemical properties can bind selectively to a biological receptor and either move it toward action or inhibit an action due to physically blocking the site from active compound binding. B) For a given molecule shape (conformation) individual atom properties can be clustered in 3D space and described numerically. C) 4-point pharmacophore with chemical properties such as charge, hydrophilicity/lipophilicity, and donor/acceptor characteristics of individual atoms mapped to 3D coordinates of cluster centers. This numerical abstraction allows researchers to use computers to predict activity of other chemicals related to specific biological function.**

(Nettles et al., 2007)

identifying the single most active compound against a specific disease or 'pathogen' when evaluating candidate formulas, botanical specimens, or synthesized chemicals. During the early discovery phase, 'activity' is measured as degree of change in the marker used to quantify the assay, irrespective of other systemic effects or overall benefit/harm to patients. A highly 'active' drug may have toxicities that pose potential harm to the general patient, but still could be used for treatments requiring toxicities acting on specifically located cell centers. An example of these are cytotoxic compounds often used in cancer treatments. Discoveries often come from serendipitous results as well as strategic sampling of candidate compounds.

As with Ehrlich's example, pharmaceutical research initially focused on natural products to find active compounds, such as arsenic, aspirin—the first patented drug discovery from a botanical specimen. Paclitaxel (marketed as Taxol®), is an important extract from the Pacific yew tree discovered using general natural-product screening methods during the 1960s that have now become standard in the industry (Wani and Horowitz 2014).

## How Pharmacologists Currently Analyze Natural Products and Sowa Rigpa Formulas

As described in the introduction, three major collaboration types occur in which pharmacologists analyze Sowa Rigpa formulas: (1) single plant or multicompound formula for active compound analysis; (2) complex compound analysis for multi-target/pathway analysis; and (3) complex compound for composition and safety analysis. While (1) and (3) still dominate research, (2) is a promising new field as drug discovery interfaces biomedical informatics and provides new insights by combining data derived from older methods with new techniques. The next sections detail some history, the analytical processes, and limitations for each type of collaboration.

### *I. Basic steps in active compound discovery & limitations assessing Sowa Rigpa formulas*

Early lock and key models helped pharmacologists standardize the drug discovery process. They developed biological assays, or 'bioassays,' to test substances in a controlled environment and standardize evaluation processes. Bioassays are applied *in vivo*—in live animal or plant, or *in vitro*—in tissue, cell, or isolated biological target to determine the specific biological activity of a given substance such as drug, compound, hormone, or enzyme.

As outlined in Table 1, the first step in drug discovery has a biological focus: to determine a biological model system that can quantifiably assay a specific compound or formulation against a specific disease model. These assays can fall into the general categories of phenotypic or targeted approaches to drug screening. Phenotypic screens are often performed in whole cell or whole organisms while looking for measurable changes such as growth, death, or other generally observable responses to drug treatment.[16] Targeted screens use specific cell types or genetically identified proteins of interest that are over-expressed, isolated/purified, or otherwise engineered into animals to test the specific hypothesis.

The second step has a chemical focus: to assemble a 'library' of potential new compounds or mixtures for testing. This is applied to discover drugs from either natural product or synthetic sources. Individual compounds are first extracted and 'purified' by a range of hydrophilic (water-loving) or lipophilic (fat-loving) chemical solvents using physical separation methods (Horowitz 1994; Wani and Horowitz 2014). Then single molecules are characterized for elemental composition, structure, and chemical properties, such as relative affinity for aqueous or lipid phases. Using these properties, libraries can be designed to either discover new leads in a phenotypic assay or elucidate the 'pharmacophore' for known active drugs through a 'targeted' screen. While a number of keys may go into a lock, only those with chemical features placed in a complementary spatial array are able to activate or deactivate a specific lock (Figure 4A). With sophisticated computational informatics methods, drug discovery programs are able to correlate these 3- and 4-dimensional chemical pharmacophore patterns with specific activity to help further streamline the discovery/optimization process (Figures 4B and 4C).

In developing a library of potential natural product compounds to screen, many single compounds may be

**Table 1. Basic Steps in Biomedical Drug Discovery**

| |
|---|
| 1. Define bioassay relevant to disease of interest |
| A. phenotype-based |
| B. target-based |
| 2. Assemble chemical library for testing |
| 3. Perform assay with library in triplicate (include controls) |
| 4. Evaluate results |
| 5. Validate in different assays |

discarded during solvent-based extraction/separation (Wani and Horowitz 2014). Different preparation and extraction methods can result in differing composition and concentrations of active (and undesired) ingredients. Accordingly, to reduce experimental variability, standard procedures for drug screening analysis within a lab are rarely changed unless a pharmacological rationale has previously been determined (Heinrich et al. 2012). However, modifying the preparation steps may be critical to better test formulas as they are traditionally used. Thus sample preparation/extraction provides an important opportunity for Sowa Rigpa practitioners to affect study design before data is collected.

The third step involves testing of the extractions in a specific assay(s) with at least three separate runs to determine deviations. Single compounds or extracted mixtures identified as 'active' above a preselected threshold are called 'hits' and validated with different or more sensitive assays. Mixtures are typically further separated into constituent molecular parts by chromatographic fractioning. Each fraction is retested in the original assay for changes in potency and further refined to determine the most active 'pure' single compound in the fraction. If the isolation procedure results in a total loss of activity, complementary action can be suspected. This would ideally direct the active complex into testing for synergy, but rarely occurs due to complexity of those experiments. This is another point where Sowa Rigpa practitioners may influence biomedical research in line with traditional teachings.

The fourth step is combining data and evaluating comparative activity/toxicity for compounds within the library across all assays used for screening relative to the control conditions. The best are selected as 'lead' drug candidates and, from evaluation of chemical structure (relative to activity), one develops a hypothesis for 'why' lead drugs behave a specific way compared to similar compounds. A better understanding of what was done in the previous three steps allows the Sowa Rigpa practitioner to help develop the testable hypotheses.

The last step is validating the leads and testing the pharmacology hypothesis in additional assays. This may include different cellular activities or different models of fluid/tissue environments the compound is expected to travel during its physiological trajectory and metabolic lifecycle.

The value of a compound or mixture identified in this five-step drug discovery process is typically measured by how high a compound scores on its ability for debilitating a pathogen or affecting the target activity verses how little it harms the cellular infrastructure and/or function of the normal controls. The safety margin is the ratio of those two potencies, known as the Therapeutic Index (TI). Those with high TI are taken down the developmental path to become drugs for humans. High toxic compounds (i.e., low TI) may still be deemed valuable to destroy cancer cells. Sowa Rigpa-specific examples of this screening approach are Wangchuk and colleagues (2011, 2012).

### *Limiting assumptions of the screening approach*

This screening model of biochemical pharmacology is based on several assumptions and has related blind spots if used alone. First, the approach assumes only specific small compounds within a specimen or formula are 'active,' and thus necessary to replicate in the final drug produced. If a compound undergoes this process and is deemed 'active' or 'inactive,' 'toxic,' or 'non-toxic,' the limitations and assumptions of the method are often overlooked. Second, the model assumes bioassay environments can replicate cellular environments similar to how the compound works in the human body and may be extrapolated as such.[17] Third, the model assumes the manner in which a drug works is by either directly debilitating a pathogen, or heightening immune or some other innate responses that directly address a pathogen or malfunctioning gene/protein. *In vitro* assays cannot exactly replicate *in vivo* environments of a patient actually being orally, intravenously or anally administered a formula, however the relative ease of analysis and a century of short-term successes has fueled this system.[18] While pharmaceutical research often ignores synergistic activities beyond the single lock-and-key model, we propose that incorporating *menjor* theory into design/analysis of new screening studies and analysis of historical data, as described above, has potential to increase relevance related to results seen in patients.

### *II. Complex compound analysis for multi-target/pathway analysis via network pharmacology*

While large-scale drug screening began as a somewhat random way of relating chemistry and biology, modern drug discovery analytics allow more rigorous understanding of disease pathways based upon decades of previous single-compound screening. In our second type of *menjor*/pharmacology collaboration chemogenomic methods allow one to rapidly relate chemical structures of many drug-like molecules to complex cellular protein-receptor networks, known as molecular signaling pathways. Pharmacophores (illustrated in Figure 4B and 4C) can be used to predict molecular targets (Nettles et al. 2006; Wang et al. 2016).

The relatively new science of network pharmacology linked with systems biology and metabolomics shifts the single drug, single target paradigm by looking at how specific medicines show additive or synergistic effect by acting on multiple targets, pathways, and micro-environments (Li and Zhang 2008; Ji et al. 2009; Schwabl and van de Valk 2019; Yuan et al. 2017). Biological pathways and networks are foundations of normal cell development, growth and function. Abnormalities in signaling pathways can result in cell function aberrations, and hence, disease. Modeling abnormalities related to changes in specific genes allows small molecule drugs and antibodies to be developed that target key points along pathways with altered protein translation that may lead to disease (Liao et al. 2016), and whose proper rehabilitation can lead to normal cell function. Such pathway recognition can provide strategic diagnostic and treatment tools, as well as analysis of traditional formulas. Well-designed new studies can begin to unveil various modes of synergistic effects and develop testable models to account for such effects (Tang et al. 2015). For example, integrating systems biology and chemical informatics through this approach can allow researchers to address limitations of single compound analysis by using more integrative methods for multicompound formulas.

A recent team, from China and Germany, used the network pharmacology approach to study anti-tumor activities from the Tibetan medicine Drébu Sum Tang (Zhao et al. 2018). They used established computational methods to correlate 'pharmacophores' of single-compounds in the multi-compound formula to specific protein targets (Wang et al. 2016), then combined literature searching, computational simulations, and statistical clustering to predict/select known gynecologic cancer targets for testing. A dried, whole-formula methanol/water extract was tested for anti-proliferation activities across the three selected bioassays. This approach allowed them to experimentally evaluate hypothetical target mechanisms, predicted by the pharmacophore mapping, and propose linked activity pathways for future studies. It also allowed testing the whole formula extract including synergistic effects that would not be present with single-compound isolates. Given this type of foundational work, future studies can compare specific single-compounds and combination results in a single experimental design to investigate nuanced synergy mechanisms of the whole formula.

It is important to realize that this network method still depends upon—and is limited by—data derived from single herb/ingredient screening methods described previously. Fortunately, for Drébu Sum Tang, and herbal formulas in general, abundant carbon-containing/organic small-molecules can be readily extracted with standard solvents, characterized, and assayed using pharmacology's purity and potency standards. Accordingly, Zhao and colleagues (2018) were able to use chemical activity data from previous natural product screening across multiple biological assays to design their study. Given chemical structures of single small molecules extracted from the individual herbs, pharmacophore tools can use historical chemical screening data to predict biological targets and aid selection of best assays to test whole formula activity. This study highlights the emerging multidisciplinary techniques needed to understand complex biopharmacology of multicompound formulas and the unique perspective on the body, body pathways, and *menjor* activities used by Sowa Rigpa. Sowa Rigpa formulas tested via *in vivo* and *in vitro* models include, among others,[19] testing the growth inhibitory property of a formula called Yukyung Karné in several cell lines to assess its anticancer properties (Choedon et al. 2014).

In Sowa Rigpa, 'purity' is achieved through processing or combining substances, and Drébu Sum Tang combines three chemically complex herbal ingredients toward a more Sowa Rigpa 'pure' and 'potent' medicine used in humans. Biomedical experiments typically begin by finding which pharmacologically 'pure' single compound or element in a formula is the most quantifiably 'potent', or active, in some number of specific screening assays. But, since serendipitous discovery of synergy-based drug cocktails for HIV in the early 90s (Barry and St. Clair 1996), biomedical methods have evolved to better understand chemical and biological mechanisms for such effects (Diallo et al. 2003).

Now 'drug cocktails' combining multiple 'pure' compounds with multiple 'targets' are standard and recognized for minimizing resistance and maintaining potency for a range of diseases through the power of synergistic combinations (Jia et al. 2009). Although methods allowing for investigation of complementarity among pharmacologically 'pure' compounds are becoming more popular (Yuan et al. 2017), the potential for understanding the therapeutic values of synergistic-design in traditional multi-compound formulas remains largely untapped.

### *III. Complex compound composition-safety analysis: differing purity-toxicity concept relations*

In our third type of Tibetan-*menjor*/pharmacology collaboration, assumptions of toxicity shape the analytical techniques employed, and understanding different concepts of 'purity' and 'potency' becomes critical. As described earlier, 'purity,' in pharmacology, is the degree to which a substance is made of only one type of element

or compound, and 'potency' is any quantitative measure of concentration-dependent activity. Therefore, from a pharmacological perspective, a 'potent' toxin is a 'pure' substance where a small amount can cause great harm to healthy animals or cells. In Sowa Rigpa, 'purity' is achieved through processing or compounding substances to offset potential harm and impart full potency or beneficial effect of the combination to a specific patient. For our last example, we contrast the study of Drébu Sum Tang introduced above (Zhao et al. 2018) with current pharmacology research of mercury-containing *tsotel.*

Unlike Drébu Sum Tang, *tsotel* is not a mixture of small organic molecules that can be easily separated, characterized, and tested. Likewise, distinct from both Drébu Sum Tang and taxol, *tsotel* does not dissolve into any standard hydrophilic or lipophilic solvent typically used for cell-based testing. Although *tsotel* can be decomposed by strong acids or combusted to quantify amounts of chemically 'pure' mercury relative to other elements, the decomposition method destroys the complex substance considered the 'purified' form by Sowa Rigpa. Accordingly, those using reductionist methods that only quantify 'pure' elemental mercury without analyzing its 'pure' compounded mercury form by Sowa Rigpa would ask if the mercury in Sowa Rigpa is a "Panacea or Problem" (Sallon et al. 2006: 405). However, a follow-up clinical study by the same researchers finds: "... mercury containing Tibetan Medicine does not have appreciable adverse effects and may exert a *possible beneficial effect on neurocognitive function"* (Sallon et al. 2017:1, italics added). Even though the study group taking high levels of mercury in the form of *tsotel* showed improved functions of attention, calculation, recall, and other measures compared to low/no-mercury controls, the authors conclude: "Since evidence of mercury as a toxic heavy metal, however, is well known, further analysis of literature on mercury use in other Asian traditional systems is highly suggested prior to further studies" (ibid: 1).

While no harm is evident from *tsotel* use in this well-designed human study, the researchers' tone of caution and distrust in the conclusion is not unfounded. Considerable historical evidence exists on toxicity from other mercury-containing compounds used as medicine. Elemental mercury, as gas, is one of the most toxic elements known (Clarkson and Magos 2006), and toxic mercury salts were a common treatment for syphilis that Ehrlich's detoxified arsenic magic-bullet 'Salvarsan' replaced. Since mercury is reactive with other elements, many thousands of organic (carbon-substituted) and inorganic (non-carbon) mercury compounds have been synthesized and tested as patentable medicines, and many have been found highly toxic in animal studies.

However, mercury compounds vary widely in their toxicities (World Health Organization 2006). 'Pure' mercury vapor, and small lipid-soluble organic mercuries, such as methylmercury ($HgCH_3$), can severely damage the central nervous system, yet the toxicity of mercury sulfide containing thimerosal used extensively as an antiseptic and preservative during the late 1900s is still a topic of scientific debate (Baker 2008). Likewise, water soluble inorganic mercury, such as mercury chloride (HgCl), can cause renal and gastrointestinal toxicity (Li et al. 2018), yet cinnabar, an insoluble mercuric sulfide (HgS) appears less toxic in most animal studies (Clarkson and Magos 2006; Liu et al. 2008). 'Pure' HgS (red cinnabar) is poorly absorbed in the gut with 1000x less neurotoxicity than methylmercury and cinnabar was not metabolized to the toxic methylmercury by human gut bacteria in recent studies (Zhou et al. 2011). However, renal toxicity may occur with long-term use (Liu et al. 2008), and HgS administered to mice parents during conception/development has been associated with neurotoxicological effects in offspring (Huang et al. 2012). Interestingly, these 'pure' HgS results are consistent with Sowa Rigpa, which also teaches that cinnabar is not to be used alone, in high doses, nor without some 'taming-purification' processing (Yeshi et al. 2018).

To prepare for cross-epistemology collaboration, questions *menjor* specialists are called to answer are: what must the pharmacologist understand about making and administering *tsotel* to investigate the potential complexed forms of mercury in *tsotel*? What unique characteristics of *tsotel* described by the tradition might inform a pharmacologist's hypothesis of chemical form related to action? Questions pharmacologists are called to answer include: What are the complexed forms of mercury in *tsotel* that are less toxic than other forms of mercury? What is the mechanism, and, what is the pharmacophore?

Several recent studies extend the standard 1-D elemental analysis toward answering these questions. Using additional techniques, including 2-D powder X-ray diffraction and others that do not chemically degrade the substance, the authors determine *tsotel* is primarily mercuric sulfide (HgS) nanocrystals, with excess sulfur and small amounts of carbon and other elements. No signal for single element mercury was found by the non-destructive analysis (Zhao et al. 2013; Yan 2007; Li et al. 2016).

Previous 3D X-ray crystallography revealed that chemically, 'pure' HgS exists as 'polymorph' having at least two relatively stable molecular crystal structure forms (Figure 5). The alpha (α) form, red cinnabar, can convert to the beta (ß) black metacinnabar at high temperatures and return to the red (α) form after cooling (Miguel et al. 2014).

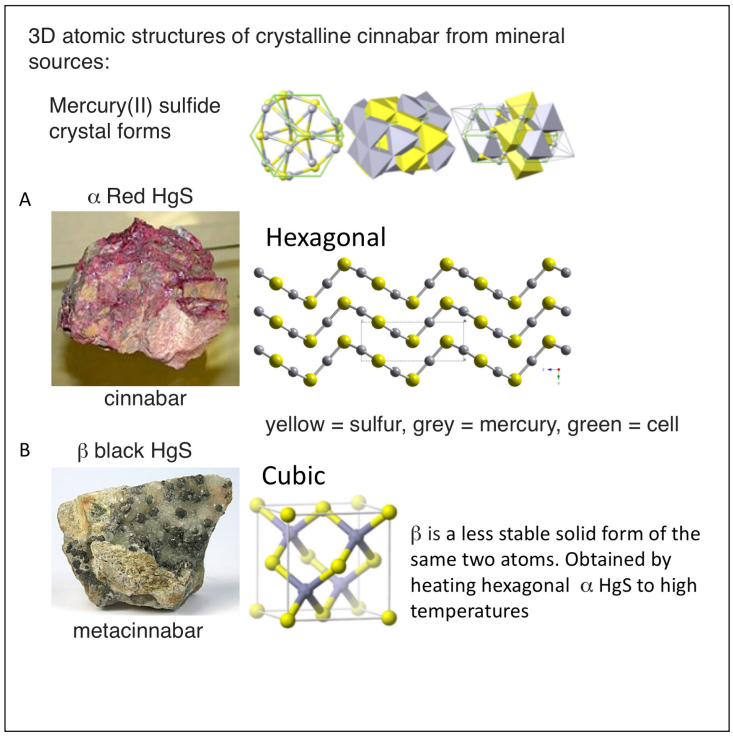


**Figure 5. HgS - Mercury (II) Sulfide Polymorphous Crystal Forms of Cinnabar determined by 3D X-ray crystallography.**
(Vladivostok, Marina. Cinnabar crystal structure. Wiki Commons, 2014: <https://commons.wikimedia.org/wiki/File:Cinnabar_crystal_structure.png>)

**A) Characterized by X-ray diffraction, α-form 'red cinnabar' is the most abundant source of mercury found on earth. Liquid HgS is precipitated by strong acids.**
(Reno, Chris. Cinnabar09. Wiki Commons, 2007: <https://commons.wikimedia.org/wiki/File:Cinnabar09.jpg>; Mills, Ben. HgS Alpha Cinnabar. Wiki Commons, 2010: <https://commons.wikimedia.org/wiki/File:HgS-alpha-cinnabar-xtal-1999-looking-down-a-axis-CM-3D-balls.png>)

**B) Characterized by X-ray diffraction, ß-form black cinnabar is a rarer, higher energy form, generated by exposing the red form to high temperatures or chemical synthesis. 'Pure' cubic HgS is unstable and will revert to hexagonal upon cooling to room temperature. Can be stabilized by organic substitution. Most abundant form identified in *tsotel* samples.**
(Lavinsky, Rob iRocks.com. Metacinnabar. WikiCommons, 2010: <https://commons.wikimedia.org/wiki/File:Metacinnabar-233443.jpg>; Mills, Ben. A zincblende unit cell. Wiki Commons, 2007: <https://commons.wikimedia.org/wiki/File:Sphalerite-unit-cell-depth-fade-3D-balls.png>)

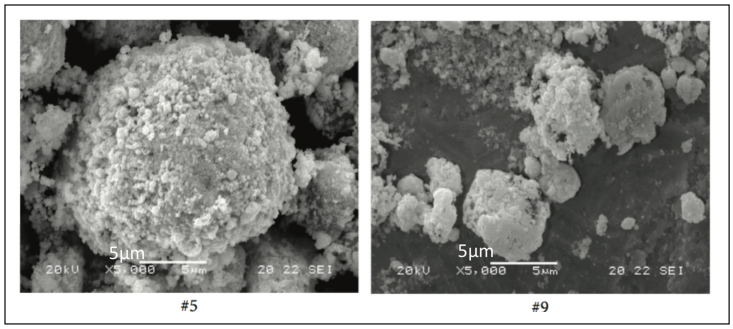


**Figure 6. Scanning Electron Microscope (SEM) of *tsotel* powder reveals clusters of ß-HgS nanoparticles ranging in size from >10 micron down to 10 nm.**

(Li et al., 2016)

These two forms may provide clues toward defining the pharmacophore for *tsotel*. A recent study comparing oral administration of *tsotel*, ß-HgS, and HgCl in mice observed kidney toxicity in HgCl-treated mice, but not in those receiving *tsotel* or pure cubic ß-HgS. The authors suggest only specific chemical species cause kidney toxicity in mice and not all mercury forms (Li et al. 2018).

Interestingly, cubic HgS forms observed in *tsotel* powder samples clustered into nanoparticles of varying size from >10 micron down to 10 nm (Li et al. 2016) (Figure 6). Nanoparticle engineering has become a growing area of pharmacology study, and unlike small-molecule drugs that enter cells by non-specific diffusion, nanoparticles only enter specific cells through selective transport mechanisms (Oh and Park 2014). Consistent with how *tsotel* in Sowa Rigpa functions similar to a *menda*, nanoparticles are now being studied as medicine carriers. However, a challenge to testing nanoparticles for activity/toxicity in cells is often lack of aqueous solubility—like *tsotel*.

To address solvation, nanoresearch often employs various carrier substances to suspend insoluble particles in aqueous media (Taccola et al. 2011). Figure 6 illustrates dispersion, micro, and nanomorphology of *tsotel* particles suspended in a solution of acacia gum and water recorded by these authors. Our observations of aqueous suspended *tsotel* as clustered nanoparticle >10 micron down to 10 nm in diameter is consistent with that seen by the previous authors in powder (Zhao et al. 2013; Yan 2007; Li et al. 2016). By sharing epistemologies in a *menjor*-pharmacology collaboration, the current authors successfully extended traditional preparation insights to modify standard analytical protocols. To characterize this important complex compound, we analyzed a pure water suspension with multiple non-destructive methods (Figure 7; Bai et al. in preparation).

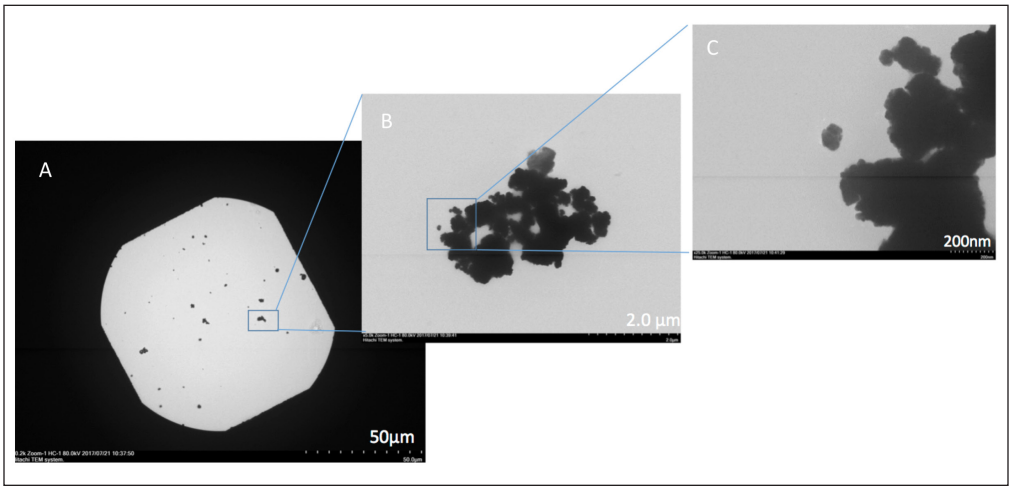


**Figure 7. Transmission Electron Microscope (TEM) images of *tsotel* nanoparticles distributed in aqueous suspension: A) Low magnification shows random dispersion of *tsotel* particles (black spots) across grid cell B) Medium magnification—spots are actually clusters of smaller clusters <100nm C) High magnification reveals large clusters are made of smaller clusters of spherical particles <50 nm in diameter.**

(Bai, Nettles, Tidwell, (c) 2017)

## Conclusion: Toward a Sowa Rigpa Model of Pharmaceutical Research

In the standard biochemical analysis approach assessing medicinal activity, the pharmacological 'purity' concept drives assessment methods. Individual, extracted compounds are screened for properties and classified by degree of toxicity, activity, and potency from a single compound 'purity' perspective. Assuming that only a few compounds in a substance are active is not a Sowa Rigpa approach to purity and potency by which combinations of single substances (and their vast multitude of molecular compounds) interact synergistically to minimize toxicity or facilitate potency. This requires the complexity of numerous molecular compound interactions to provide 'pure,' or beneficial, medicinal products. However, such reductionist methods are foundational to collect the data needed for further biochemical research on synergy.

As seen in the second analytic approach, network pharmacology, systems biology, and metabolomics have vastly improved methods for assessing potential complementary mechanisms in multi-compound systems, such as in Sowa Rigpa formulations. While analysis of constituent parts can demonstrate specific activity mechanisms, more analysis needs to focus on whole formula complexes as done for Drébu Sum Tang. As the academic sector does with synthetic pharmaceuticals, chemical library databases, derived from screening of Sowa Rigpa plants and formulas, can potentially facilitate derivation of chemical shape-based pharmacophores that can help explain active chemical families described by taste, post-digestive taste, potency, and quality profiles based in combinatorial elemental dynamic properties. Such systems biology approaches are inherently limited by assumptions made during underlying data collection (Scheid 2016) and thus require expert performance assessments. Sowa Rigpa partners can serve this critical role by ensuring extraction solvents/methods resemble those traditionally used,[20] suggest hypotheses for potential differences due to extraction methods, advise functional evaluation targets, and serve as gatekeepers to protect traditional knowledge.

The third collaboration type of composition-safety analysis of complex compounds emphasizes the importance of assessing structure to determine toxicity. This example most clearly illustrates the distinctions in concepts of 'purity' between pharmacology and *menjor*. *Tsotel* is an organo-metallic ornamented mercury sulfide nanoparticle considered 'pure' on the Sowa Rigpa side because it is made non-toxic, whereas the Euroamerican side assumes 'toxicity' based on the amount of chemically 'pure' mercury, not the actual form given to patients. A recent study conducted by Liu and colleagues (2018) propagates this misconception of mercury toxicity irrespective of conjugated forms (such as mercury sulfide) into its models of environmental pollution in Tibet from mercury, which they attribute to the inclusion of *tsotel* in precious pills consumed in Lhasa. The study highlights the purity paradigm used by biomedically-trained chemists regarding toxicity, as Liu and colleagues rely upon a destructive method to evaluate amount of all elemental mercury (Liu et al. 2018: 8839) and did not test for stable non-toxic forms (Clarkson & Magos 2006; Liu et al. 2008; Zhou et al. 2011; Morais et al. 2012; Li et al. 2016). Additionally, they greatly overestimate the quantity and frequency of precious pill consumption across Tibetan populations.

Given studies in animals showing effects in promoting sleep, relaxation, reducing fever (Zeng et al. 2005; Jiang et al. 2009), enhancing immunity, inhibiting expression of caspase-3, reducing inflammation, and extending life in fruit flies (Dorje and Lobu 2008; Chen et al. 2011; Zhu et al. 2013), and *tsotel*'s lack of toxicity in humans (Sallon 2017), whole-formula follow-up studies in human cell lines to determine mechanism are warranted. However, difficulty

of suspending *tsotel* particles in homogenous solutions has inhibited cellular studies, as were done for Drébu Sum Tang. Therefore, new methods for safety/potency testing of such important substances in their Sowa Rigpa 'purified' nanoparticle forms are still needed.

In conclusion, we propose engaging the distinct epistemologies of different intellectual traditions by recognizing key concepts such as the 'purity'/'potency' paradigms presented here. Such engagement, can generate new understandings of 'synergy-by-design' for pharmacology and a greater appreciation of unique chemical structures and formulas for *menjor*.[21] Accordingly, highly trained Sowa Rigpa *menjor* partners in collaboration with interdisciplinary pharmacological teams can help design experiments validating significant *menjor* developments by encouraging pharmacology experts to explore more synergy-directed techniques and analysis beyond the single lock/key model when studying Sowa Rigpa formulas.

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## Endnotes

1. Known as *menpa*, *lhajé,* or *amchi* (*sman pa, lha rje, am chi*).

2. Prominent commentaries for the discussion here include: Kyempa Tsewang (2000 (15th cent.)); Zurkhar Lodrö Gyalpo (1989 (16th cent.)); Deumar Tenzin Püntsok (2009 (17th cent.)); Desi Sangyé Gyatso (1994, 2005 (17th cent.)); Kongtrul Yönten Gyatso (2005 (19th cent.)); Troru Tsenam (2000 (20th-21st cent.)).

3. Initiating from Tibet, Sowa Rigpa traditions spread to and developed in Mongolia, Bhutan, Ladakh, Nepal, Buryatia, Tuva, Kalmyk, and other Tibetan culturally-influenced regions. They share *materia medica* comprising a wide breadth of minerals, gems, precious substances, flora, and fauna with regional particularities for locally procured specimens and similar sources for those distally traded. Traditions regionalize to local ecologic, social, cultural, philosophical, and epidemiologic contexts. Disease theory and treatment approaches rely on systems linking organs, fluids, bodily constituents, processes, and activities described by specific functional characteristics linking related activities modified by diet, behavior, medicine, and therapeutic interventions. The *Four Tantras* and its most prominent commentaries structure Sowa Rigpa theory and praxis still used today of etiology, diagnostics, and treatment applying these functional characteristics of body physiology, material substances, and therapeutic properties.

4. Sowa Rigpa *menjor* theory stems primarily from Chapters 19-21 of "Explanatory Tantra," and Chapters 3-12 of "Subsequent Tantra" on compounding distinctions in the *Four Tantras*. Other important theoretical contributions include: Chapters 1-3 and 5 of "Root Tantra;" treatment sections of entire "Oral Instructions Tantra;" Chapters 13-19 of "Subsequent Tantra," and relevant external therapies sections (Chapters 20-25).

5. Notable examples include anti-hypoxic activities identified from *Arenaria kansuensis* (Cui et al. 2018), used for altitude sickness in Sowa Rigpa; immunomodulatory properties exhibited by five Sowa Rigpa plants emerging as frontline treatment agents for cancer, infectious disease and autoimmunity (Wangchuk et al. 2018); as well as antimalarial activity identified against a multidrug resistant *Plasmodium falciparum* strain from another Sowa Rigpa plant (Wangchuk et al. 2013).

6. Another example is Sowa Rigpa-derived formulas tested for the modulation of advanced glycation end products and advanced oxidation protein products in bovine serum albumin as a model protein (Grzebyk and Piwowar 2014).

7. Primarily from the work of Indian Buddhist logicians Dignāga and Dharmakīrti after the 7th century CE.

8. Even physiological pathways and activities in Sowa Rigpa that, in their default mode, link body constituent, organ, fluid, and energetic signaling dynamics to provide systemic functions, relate to these elemental dynamics. These psychophysiologic default systems are called the three *nyépa* (*nyes pa*) or *rlung* (*rlung*), *tripa* (*mkhris pa*), and *béken* (*bad kan*) responsible for functions of motility and psychophysiologic signaling; metabolic heat, blood production, and thermoregulation; and fluid-nutrient cycling, filtration, joint lubrication, and solidity/cohesion, respectively. *Rlung* exhibits properties of the elemental wind dynamic, *tripa* the elemental fire dynamic, and *béken* the elemental earth and water dynamics. The Tibetan term *nyépa* refers to their activity as the primary instigators of disease and imbalance in the body, like a weakness that befalls the Achilles heel and results in systemic debilitation. Although the standard phonetic conversion (Germano and Tournadre 2010) for *rlung* is '*lung*' (pronounced *lōōng*), we retain the Wylie spelling '*rlung*' to distinguish the term and prevent confusion with the organ lung.

9. Subcategories of the three *nyépa* physiological systems named for their respective primary function.

10. For example: medicine composed of an earth dynamic is heavy, stabilizing, dulling, smoothing, oiling, yet dry in quality; often aromatic, and stiffening, bulkening, cohering in functional activity. Due to interaction with characteristic properties of *nyépa* pathways, earth-dominant medicine pacifies *rlung* and increases *béken*.

11. Though narrower in scope than Euroamerican herb classes.

12. Known as the 'Seven Essential Practices for Cultivating Medicinal Quality' ('*sman la gces par 'os pa'i yan lag bdun*), comprising proper: (1) collection location, (2) collection season and time of day, (3) removal of harmful and toxic components, (4) drying location according to warming and cooling principles, (5) storage and suitable shelf life, (6) smoothing of characteristics, and (7) compounding according to specific desired properties of elemental dynamics, tastes, post-digestive taste(s), potencies, qualities, and various other characteristics (Yuthog Yönten Gönpo 2008: 696-700). For commentarial elaboration, see Deumar Tenzin Püntsok (2009: 458-466).

13. We use 'potentiate' to gloss: "Drying and sorting according to its own potency imbues immeasurable qualities" (*rang gi nus ldan yon tan dpag tu med*) (Yuthog Yönten Gönpo 2008: 697).

14. Sowa Rigpa traditions can vary in these practices (e.g., Boesi (2006); Dorje and Lobu (2008)).

15. For related discussions, see Yang Ga (2014) and Tidwell (2017).

16. For example, taxol was one of the first compounds isolated by government-funded natural product extraction, and found to reduce abnormal growth of cancerous tumors in mice during the 1960s. Later, in the 1990s, targeted screens focused on determining how (Wani and Horowitz 2014).

17. Recent work by Klein and colleagues (2013) attempts to minimize this limitation through pathway-focused bioassays and transcriptome analysis.

18. Funding mechanisms tend to drive this research approach through supporting related infrastructure, equipment, and analytical methods.

19. Jenny et al. (2005); Vennos et al. (2013); Radomska-Leśniewska et al. (2013); Grzebyk & Piwowar (2014).

20. Such as oil, boiled-water, ethanol, and so forth.

21. Bhutan healthcare policy (since 1967) placed Sowa Rigpa practitioners alongside pharmacological researchers to develop integrated scientific, quality control, and safety/efficacy protocols (Wangchuk and Tashi 2016). Several collaborations between top Tibetan medical institutes in India and Tibet also used *menjor*/pharmacology partnership approaches, such as Choedon et al. (2014) and Schinazi and Dawa (2010). However, understanding unique *menjor* aspects of synergy in Sowa Rigpa has not been a direct focus of these past collaborations. This article aims to contribute toward such collaborative endeavors going forward.

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