— **Review** —

Overview and Frontier for the Development of Metallopharmaceutics

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This review introduces the development of metal-containing pharmaceutics (metallopharmaceutics) such as anticancer agents containing platinum (Pt) and ruthenium (Ru), and superoxide dismutase (SOD) mimetic, focusing on the recent topics on antidiabetic vanadium (V) and zinc (Zn) complexes as well as antioxidative copper (Cu) and Zn complexes. From the ancient ages, people used many types of inorganic compounds to treat physical disorders or diseases. Since the modern concept of chemotherapy was achieved by Paul Ehrlich, who developed the arsenic (As)-containing compound to treat syphilis in 1910, a wide variety of metallopharmaceutics have been proposed and clinically used worldwide. This review is described for the researchers who are interested in the current states for the development of metallopharmaceutics.

Key words —— metallopharmaceutics, inorganic compound, chemotherapy, anticancer, antidiabetes, antioxidative

INTRODUCTION FOR METALLOPHARMACEUTICS

In the prehistoric times, humans have been exposed to metals, which existed in the pure states as gold (Au) and mercury (Hg) or in the ionic states as copper (Cu) and iron (Fe) in nature. The first finding of metals must date back thousand years (Table 1). During the prehistoric and middle ages, 9 and 5 elements have been found and used, respectively. Discovery of metals and elements from nature increased in the 18th century, which followed in the 19th century making the maximum in the numbers (Table 2).[1)] At the end of 19th century, radionuclides were found. In addition, the transuranic metals, that have higher atomic number than uranium (U), appeared on our planet in 1940's by the discovery of nuclear fission.

The first finding of an element from human urine was due to the German alchemist Henning Brand (1639–1710), who discovered phosphorous (P) in 1669. This was a significant example for the finding of element originated from living materials. Following the finding, vitamins, bio-trace elements and hormones have been detected, and in 20th century a wide variety of metalloproteins and metalloenzymes have been found and then actually isolated from bacteria, cultured cells, animal and human organs and blood.

Table 1. Use of Metals in Ancient Age

Au and Ag	4000 B.C.	Chaldear
Pb	3800 B.C.	
Cu and Sn	3500 B.C.	Bronze Age
Fe	1500 B.C.	Iron Age
Hg and As	350 B.C.	Ancient Greece
Zn	—	Ancient Romans

Inorganic compounds have empirically been used to treat human and animal disorders or diseases; for instance, Hg was used for treating syphilis, magnesium (Mg) salts for intestinal treatments, and Fe salts for treating anemia. Such metal-containing compounds in ancient times were naturally crude preparations obtained from minerals, plants, and animal sources.

Through the time of Paracelsus (1493–1541),

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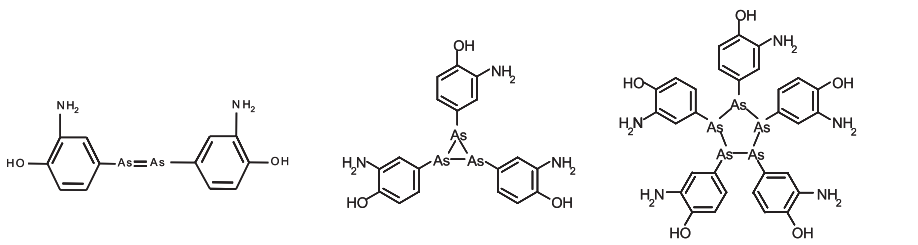
Table 2. Elements Discovered from Nature

Age	Number	Element
Prehistoric Age	9	Au, Ag, Hg, Pb, Sn, Cu, Fe, S, C
Middle Age	5	Zn, As, Bi, Sb, P
18th Century		
the first half	3	Co, Ni, Pt
the latter half	14	Mn, W, Ti, Mo, Cr, U, Zr, Y, H, N, O, Cl, Te, Be
19th Century		
the first half	27	Mg, Pd, Os, Ce, Rh, Ir, Na, K, Ca, Sr, Ba, Li, Cd, Se, Si, Ta, Al, La, Th, V, Er, Tb, Nb, Ru, B, I, Br
the latter half	25	Rb, Ce, Tl, In, Ga, Yb, Sc, Sm, Ho, Tm, Gd, Ge, Pr, Nd, Dy, Po, Ra, Ac, F, He, Ne, Ar, Kr, Xe, Rn
20th Century	5	Eu, Lu, Pa, Hf, Re



The syructure proposed by Ehrich and his co-workers

Recent study revealed that Salvarsan was a mixture of cyclic species

Fig. 1. Strucures of Salvarsan[2)]

who proposed the application of several inorganic pharmaceutics containing Hg, Sb, Pb, Cu, arsenic (As), S, B, and Ag to treat diseases, interest in inorganic pharmaceutics has slowly risen in European countries.

The great advance of medical use of inorganic compounds came in 1910. Paul Ehrlich (1854–1915) and his co-workers discovered arsphenamine "Salvarsan," which is an organo-metallic compound with inorganic arsenic-carbon (As-C) bond and spirochaeticidal activity. This compound brought not only the concept "chemotherapy" but also the methodology to develop a wide range of As compounds for the treatment of syphilis. Salvarsan was actually the first artificially prepared and clinically used pharmaceutic with inorganic element, As (Fig. 1).[2)]

Although Ehrlich and his co-workers proposed that Salvarsan contains an As=As moiety, recent modern chemistry revealed that the true structures of Salvarsan is a mixture of cyclic As-As bonded species (Fig. 1). The newly proposed trimer and pentamer slowly release $RAs(OH)_2$ (R=3-amino-4-hydroxyphenyl) species, giving rise to Salvarsan's antisyphilis activitiy.[2)]

On the other hands, Alfred Werner (1866–1919) proposed the "coordination theory,"[3)] and a new concept of "metals and life" came into being with the finding of urease, which is the first crystallized enzyme obtained in 1926 and found to bind nickel (Ni) at the active center in 1975. Approximately thousands of metalloenzymes and metalloproteins are now crystallographically analyzed.

With the development of both analytical chemistry and keeping-method of experimental animals, the concept of essential trace elements in humans and animals has been established, and health disorders due to deficiency of elements such as Fe, Zinc (Zn), Cu, and Se in humans has been observed in several countries. In most cases, the supplementa-

Table 3. Established and Potent Metallopharmaceutics

Element	Year	Treatment	Compound
Ag	1974	Burn and wound dressings	Silver-sulfadiazine
			Slow-release Ag compounds
Al	1968	Stomach ulcer	Sucralfate
Au	1960	Rheumatoid arthritis	Auro-thiolamt
			Auro-thioglucose
			Auranofin
As	1910	Syphilis	Arsphenamine[#]
		Acute promyelocytic leukemia (APL)	Trisenol (Arseno trioxide)
Bi	2009	*Helicobacter pylori*	Bismuth sulfosalicylate
Co	1940	Growth delay, Anemia	Cyanocobalamin
Cu	2007	Antioxidant, UV-induced dermatitis	Copper-asprinate[*,#]
Ge	1985	Bacteria, Cancers	Ge-132[*]
Li	1954	Manic depressive psychoses	Lithium carbonate
Mo	1981	Wilson disease	Tetrathiomolybdate[*]
Pt	1969	Cancers	Cisplatin[#]
Ru	1953	Cancers	Chlorido-ammine Ru
Se	1998	Acute ischemic stroke	Ebselen[*]
V	1990	Diabetes mellitus	Vanadium complexes[*,#]
Zn		Stomach ulcer	Polaprezinc
	2002	Diabetes mellitus	Zinc complexes[*,#]
	2003	UV-induced dermatitis	Zinc complexes[*,#]
	2007	Metabolic syndrome	Zinc-thioallixin-*N*-methyl[*,#]
		& Diabetes	Zinc-dithiocarbamate[*,#]
	2008	Wilson disease	Zinc-acetate[#]

[*]anticipated to be developed as pharmaceutics. [#]will be described in the review.

tion of these elements alleviated disorders in human and animal health.

On the basis of these backgrounds, a wide variety of metal-containing pharmaceutics, metallopharmaceutics, have been proposed, and some of them have been clinically used since the 20th century, as shown in Table 3. In this review, some metallopharmaceutics (isolated metal complexes or coordination compounds with therapeutic effect and potential) will be described.

SEVERAL METALLOPHARMACEUTICS

The most well-known example for the use of coordination compounds in the treatment of diseases is platinum (Pt) complexes in cancer therapy. In 1965, Barnett Rosenberg (1926–2009) and his coworkers observed the unusual phenomenon of filamentous growth of *Escherichia coli* bacteria. This metamorphosis was induced by Pt^{2+} and Pt^{4+} ammine chloride complexes, which were generated *in situ* during electrolysis at the Pt electrodes, because the medium contained ammonium chloride. The compound responsible for the filamentation was *cis*-diamminedichloroplatinum(II) (cisplatin), which is a classic coordination compound that was first prepared in 1844. Cisplatin has been found to be highly effective to treat cancers in not only animals but also humans with testicular, ovarian, lung, bladder, heart, neck, and cervical cancers (Fig. 2).[4–6] The mechanism of anticancer activity of cisplatin has been actively studied for many years (Fig. 3),[7] however, it is not yet fully understood. In recent years, new Pt complexes of the second and third generations of cisplatin and polynuclear Pt complexes, aiming at acting against cisplatin-resistant tumors, have been proposed (Fig. 4).[4–6]

Surprisingly, anticancer activity of ruthenium (Ru) complexes was reported by Dwyer and coworkers in 1952. However, the research has been forgotten by the serendipitous and brilliant finding of cisplatin in 1962. In the beginning of the research, monodentate ligands to Ru were used, however, in recent years more complicated ligands have been used to enhance the anticancer activity (Fig. 5).[8]

Reactive oxygen species (ROS) have been

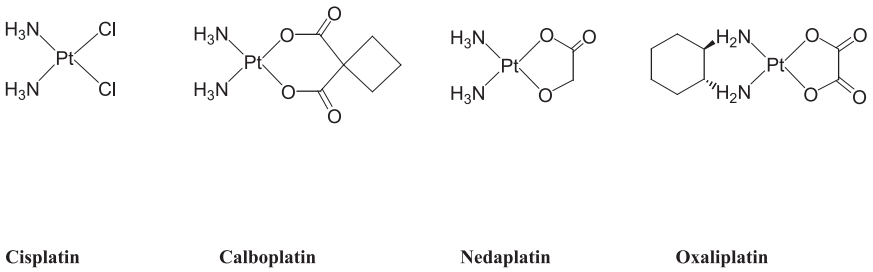


Fig. 2. Anticancer Pt Complexes Currently Used in Japan[4–6)]

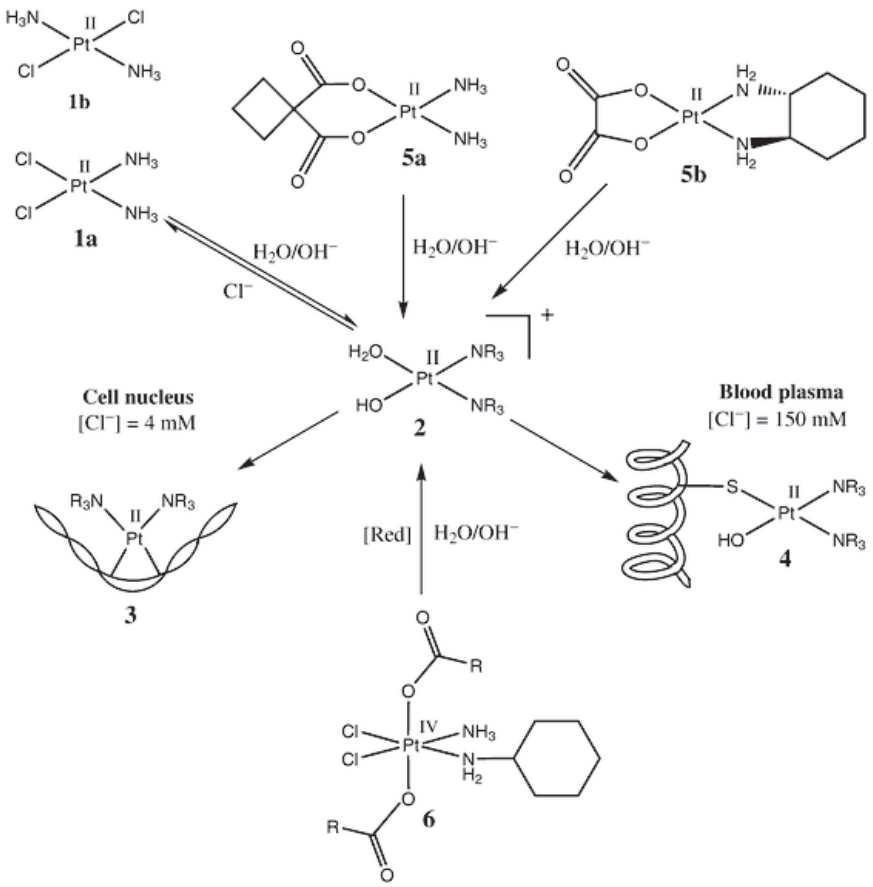


Fig. 3. Proposed Mechanism for the Action of Anticancer Pt Complexes[7)]

known to be involved in the pathogeneses of various diseases such as lifestyle-related diseases, hypertension, and photo-aging due to exposure to ultraviolet (UV) light exposure. Cu-dependent and Zn-modulated cytosolic and extracellular superoxide dismutases (Cu_2Zn_2SOD) exist in a wide range of mammals, and catalyze the dismutation reaction of superoxide anion radical ($\cdot O_2^-$) to yield hydrogen peroxide (H_2O_2) and triplet state oxygen ($^3O_2$). SOD activity in mammalian cells tends to decrease with an increase in age, the intake of SOD activity-enhancing compounds, which can facilitate *de novo* synthesis of Cu_2Zn_2SOD, is thus recommended. Because SOD is easily digested by gastric and intestinal proteases, the importance of oral intake and skin ointment of small-molecular-mass SOD-mimetic metal (Cu, Mn, and Fe) complexes with active site structures similar to those in SOD enzymes has been proposed since 1974,[9,10)] and research on the development of SOD-mimetics has been performed by many researchers (Table 4).

Among the many metallopharmaceutics involv-

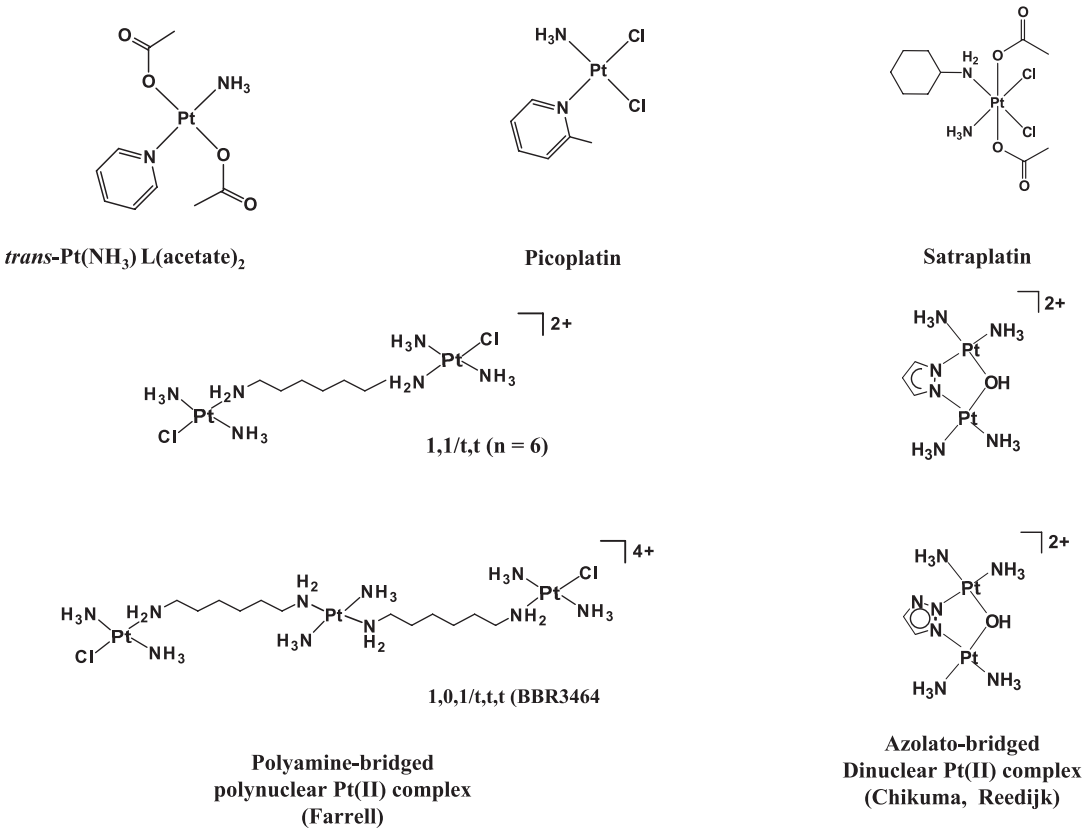


Fig. 4. Potent Anticancer Pt Complexes[4–6)]

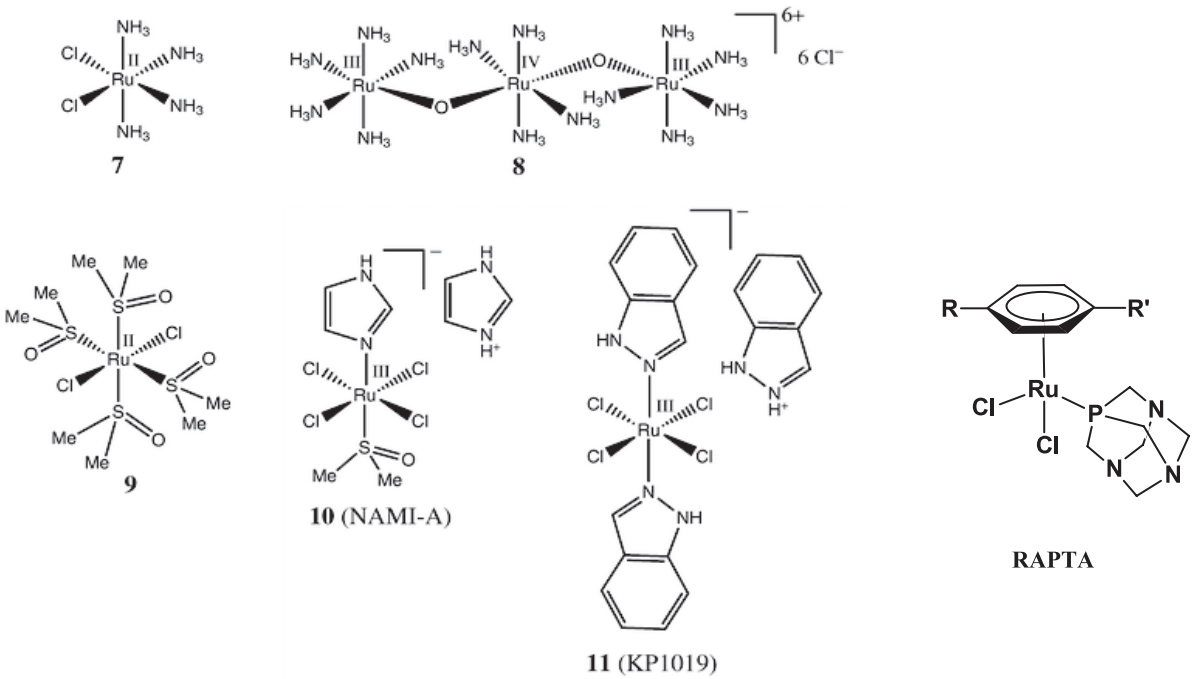


Fig. 5. Potent Anticancer Ru Complexes[8)]

Table 4. SOD Mimetic Complexes

Year	Author	Complex
1974	Brigelius *et al.*	$Cu(lys)_2$
1976	Sorenson	$Cu(3,5\text{-diisopropyl salicylate})_2$
1978	Younes *et al.*	$Cu(salicylate)_2$
1983	Goldstein *et al.*	$Cu(o\text{-phen})_2$
1986	Kimura *et al.*	Cu(cimetidine)
1987	Darr *et al.*	Mn^{4+}-DFO
1989	Nagano *et al.*	Fe^{2+}-TPEN
1993	Itami *et al.*	$Cu(pyrimine)_2$
1995	Fragoso *et al.*	Mn, Cu-β-cyclodextrin with dithiocarabmate
1999	Yamato *et al.*	Mn(*N*,*N*-bis(pyridylmethyl)-S-histidine)
2000	Ohtsu *et al.*	Cu, Zn(bdpi)
2001	Jitsukawa *et al.*	Cu_2(tppen)
2002	Tang *et al.*	Mn-schiff base
2003	Abdel *et al.*	V, Mn, Fe, Co, Zn-2methylaminopyridine
	Abou-Self *et al.*	Cu-2methylaminopyridine
2005	Fujimori *et al.*	$Cu_2(aspirin)_4$
2007	Marinko *et al.*	Mn(porphyrin)
2009	Serbest *et al.*	Fe, Co, Ni, Cu, Zn-Schiff base

ing a variety of metal complexes (Table 3), recent developments in potent antidiabetic vanadium (V) and Zn complexes as well as anti-oxidative metal complexes will be focused in this review.

BIOLOGICAL ROLES OF SOME METALS

Vanadium

Vanadium (V) is a late-comer in the field of "life and metals." In 1977, V biochemistry was opened when vanadate (+5 oxidation of V) was identified to inhibit sodium and potassium ATPase (Na^+-K^+-ATPase), because V was contaminated in an ATP preparation derived from equine muscle.[11] Subsequently, the inhibition was proposed to have been caused by the substitution of phosphate with vanadate in ATP-driven reactions. This finding recognized the importance of V biochemistry and stimulated studies on V in many enzyme systems involving adenylate cyclase, tyrosine kinase, phosphotyrosyl phosphatase, and ribonuclease. V has been revealed to exhibit a wide variety of biological functions (Fig. 6).

Among the biological roles of V, the insulinomimetic effect is the most striking; this effect is provided by the oxidation states +3, +4 (vanadyl, VO^{2+}), and +5 (vanadate, VO_3^- or VO_4^{3-}). One of the current focuses is to create pharmaceutics that will take advantage of the insulinomimetic and antidiabetic properties of V in the place of insulin injections and synthetic drugs.[12–14] Interestingly, before the discovery of insulin in 1922 by Banting and Best, French physicians reported in 1899 that sodium metavanadate ($NaVO_3$) partially improved the state of human patients with diabetes mellitus (DM).[13, 14] In recent years, both $VOSO_4$ and $NaVO_3$ have been clinically examined to improve human DM. In addition, $VOSO_4$ is known to be less toxic to rats than vanadate compounds,[15] and most V in the organs of normal rats treated with vanadate is exclusively present in the vanadyl form. From these observations, it followed that low-molecular-weight ligands for vanadyl could be used with the expectation of obtaining higher bioavailability and lower toxicity than vanadyl or vanadate alone in animals.[13, 14]

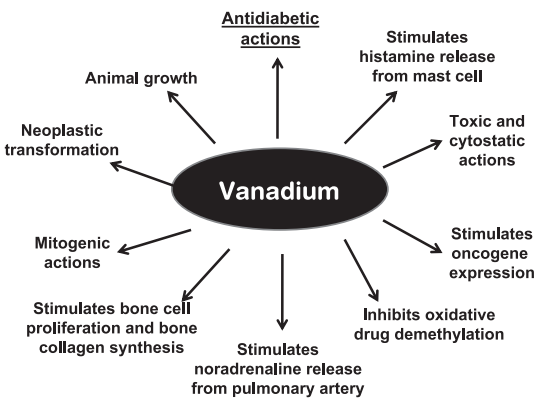


Fig. 6. Biological Roles of Vanadium

Zinc

There are now approximately 200 three-dimensional structures for Zn proteins, representing all classes of enzymes and covering a wide range of phyla and species. Zn proteins and enzymes function as structural, catalytic, and cocatalytic factors. Unique Zn proteins were discovered in the 1980s. The first transcription factor found in 1983 was identified as a Zn enzyme, leading to the DNA-binding finger protein in 1985.[16] Many proteins possess a Zn-containing motif that serves to bind the DNA embedded in their structure. In 1995, a Zn transporter, which participates in a homeostatic system in cell, was discovered. Metallothionein (MT), which was discovered in 1957, was found to link Zn distribution in cells in 1998.[17] In 2000, a Zn-containing regulatory protein was found to have a role in neurotransmission.[18]

One of the major advances was the discovery of a homeostatic system of proteins that controls cellular Zn by coordinating Zn import and export, distribution, and sensing of Zn status. The involvement of many proteins in homeostatic control increases the potential for variations in Zn metabolism due to mutations in these proteins.

The biological roles of Zn in the cells and organs are summarized in Fig. 7. Zn is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis. Zn functions in immunity, intermediary metabolism, DNA metabolism and repair, reproduction, vision, taste, and cognition behavior. In addition, Zn is essential for neurogenesis, synaptogenesis, neuronal growth, and neutrotransmission.

Currently, antidiabetic activity of Zn is important. Zn and DM are linked at several points during metabolism in a cell.[19] Zn is contained in insulin and it binds with three nitrogen atoms from His and three H_2O molecules in an irregular octahedral environment.

Zn was found to stimulate rat adipocyte lipogenesis similar to the action of insulin, which followed by observations of the *in vivo* antidiabetic effects of oral $ZnCl_2$ in streptozotocin-induced type 1-like diabetic rats (STZ rats) and obese (ob)/ob mice in 1992[20] and 1992,[21] respectively. However, in these observations, high doses or long-term administrations (8 weeks) of Zn were used, owing to the low bioavailability of $ZnCl_2$. Then, the coordination compounds of Zn were recommended in order to enhance its bioavailability and therapeutic potential. The first orally active antidiabetic Zn complexes were discovered in 2002. Since then, a wide variety of insulinomimetic Zn complexes with different coordination environments around Zn has been proposed.[13, 14, 19, 22]

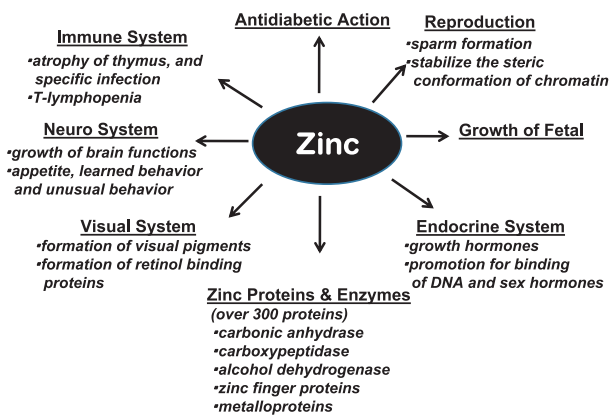


Fig. 7. Biological Roles of Zinc

Copper

Copper (Cu) is essential in all plants and animals. Cu distributes widely in human body occurring in the liver, kidney, muscle and bone. The metal is found in a wide range of proteins and enzymes involving the catalytic active center of cytochrome c oxidase, superoxide dismutase, and blue Cu proteins.[23] In the blue Cu proteins such as azurin and plastocyanin, Cu participates in electron transport.[24] In addition, Cu participates in the oxygen carrier similarly to Fe in hemoglobin and myoglobin in mammals. Some mollusk and arthropod have the high molecular weight Cu pigment, homocyanin. When the proteins are oxygenated in the blood, they turn blue color.[25]

Cu and Zn sometimes compete for the absorption in the digestive tract, thus a diet that is excessive in one of these metals may result in a deficiency in the other. The symptoms of Wilson's disease are caused by accumulation of Cu in the liver. Recently, Zn acetate has been revealed to treat the disease by lowering Cu absorption from the digestive tract (Table 3).[26]

An important role of Cu is in facilitating Fe uptake in humans. Cu deficiency often induces anemia-like symptoms.[27] Biological roles of Cu are summarized in Fig. 8.

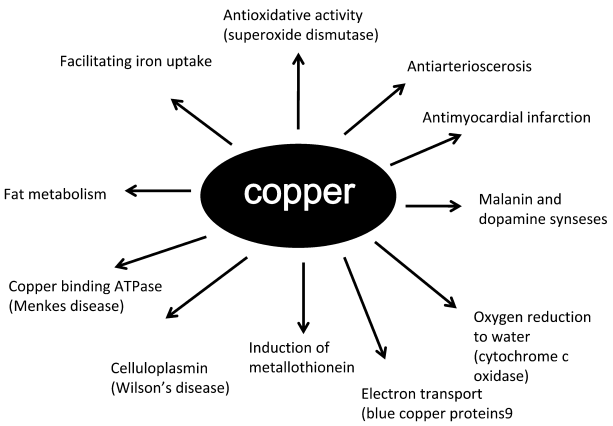


Fig. 8. Biological Roles of Copper

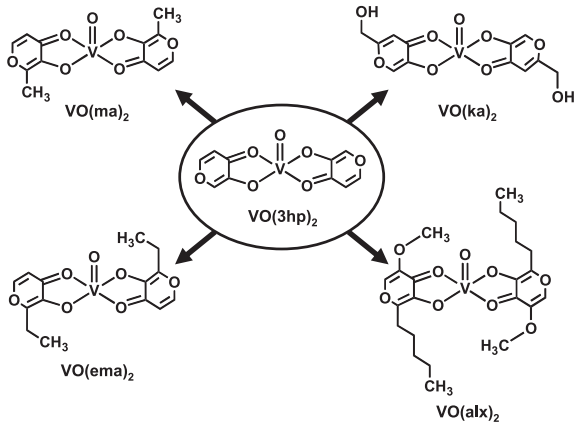


Fig. 9. Chemical Structure of $VO(3hp)_2$ and Its Related Complexes[32)]

ANTIDIABETIC METAL COMPLEXES AND ACTION MECHANISM

Vanadium

The insulinomimetic effect of V ions on cells and diabetic model animals has been reported since the 1980s. V ions and their complexes exert various insulinomimetic and antidiabetic effects involving the enhancement of glucose transport and metabolism in isolated adipocytes and hepatocytes as well as skeletal muscle, stimulation of glycogen synthesis and lipogenesis, inhibition of lipolysis, and protein metabolism. Because inorganic V ions are less absorbed from the digestive tract (low bioavailability), the complexation of V ions with organic ligands is a useful method for reducing toxicity and improving both bioavailability and tissue uptake of the ions. Among the three oxidation states of V ions, oxovanadium(IV) form (vanadyl) has the advantage of exhibiting insulinomimetic and antidiabetic activities in terms of stability in cells and toxicity and efficacy in animals. Thus, a large class of vanadyl complexes has been extensively proposed by many research groups (Table 5).[13, 14, 19, 22, 28)] The first generation of vanadyl complexes such as bis(methylcysteinato)oxovanadium(IV) $[VO(cysm)_2]$ (1990),[29)] bis(maltolato)oxovanadium(IV) $[VO(ma)_2]$ (1992),[30)] and bis(picolinato)oxovanadium(IV) $[VO(pa)_2]$ (1995)[31)] normalized hyperglycemia (high blood glucose level) in STZ rats.

To find more potential vanadyl complexes than the first generation of vanadyl complexes, a comprehensive study has been performed. For instance, the *in vitro* and *in vivo* structure-activity relationships of bis(3-hydroxy-4-pyronato)oxovanadium(IV) $[VO(3\text{-}hp)_2]$ related complexes with a $VO(O_4)$ coordination environment were examined. From the study, the second generation of vanadyl complexes such as bis(ethylmaltolato)oxovanadium(IV) $[VO(ema)_2]$ and bis(allixinato)oxovanadium(IV) $[VO(alx)_2]$ were discovered (Fig. 9).[32)]

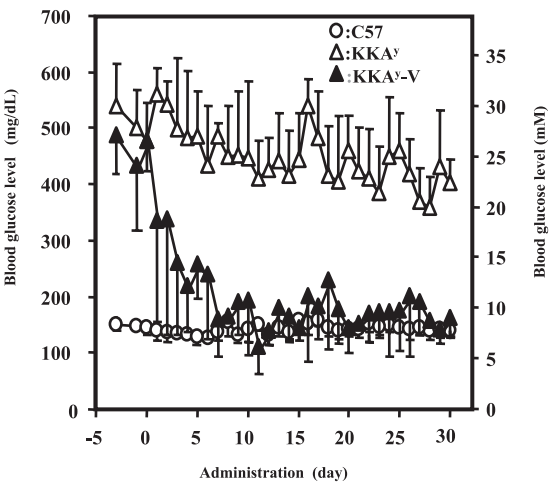
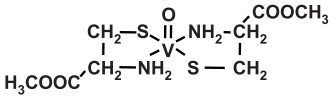
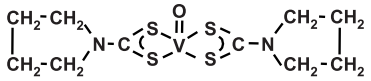
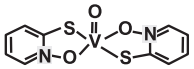
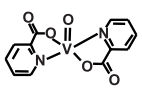
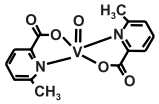
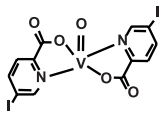
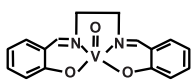
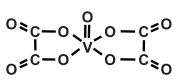
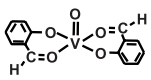
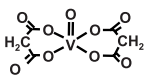
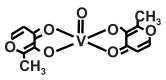
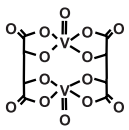
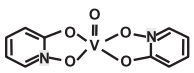
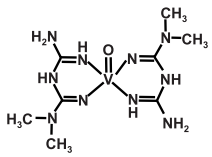
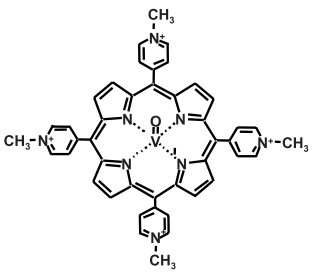
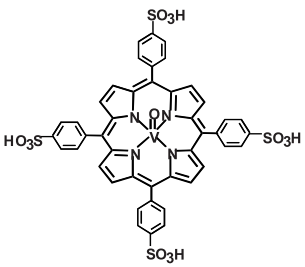


Fig. 10. Blood Glucose Level in Normal Mice (C57/BL) and KKA^y Mice after Daily Oral Administration of Saline and $VO(alx)_2$, Respectively, for 30 days[32)]

Doses were adjusted to maintain a concentration of 3–7 mg V kg^{-1} body weight based on daily changes in the blood glucose level. Data are expressed as the mean and S.D. for 4–6 mice.

When the $VO(alx)_2$ complex was orally administered to KK strain transformed A^y allele by repetitive back crossing (KKA^y) mice, which are obesity-linked type 2 DM model animals similar to human DM, for 4 weeks, an improvement was observed in both the blood glucose (Fig. 10) and hemoglobin $(Hb)A_{1c}$ levels. In oral glucose tolerance test (OGTT) after the treatment period, the

Table 5. Typical Antidiabetic Vanadyl Complexes with Different Coordination Environments[13, 14, 19, 22, 28)]

N_2S_2						
	$VO(cysm)_2$					
S_4						
	$VO(pdc)_2$					
S_2O_2						
	$VO(opt)_2$					
N_2O_2						
	$VO(pa)_2$	$VO(6mpa)_2$	$VO(5ipa)_2$	VO(salen)		
O_4						
	$VO(ox)_2$	$VO(sa)_2$	$VO(ma)_2$	$VO(mal)_2$	$(VO)_2(tar)_2$	$VO(opd)_2$
N_4						
	$VO(metf)_2$	VO(TMpyP)	VO(TPPS)			

blood glucose levels of the $VO(alx)_2$-treated KKA^y mice was almost equivalent to that in normal mice at 15 min after glucose loading. Plasma hyperinsulinemia in KKA^y mice was also completely normalized by the $VO(alx)_2$ treatment. These results indicate that $VO(alx)_2$ treatment improves insulin resistance and glucose intolerance in KKA^y mice.

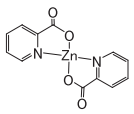
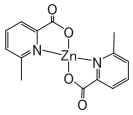
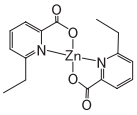
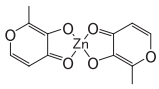
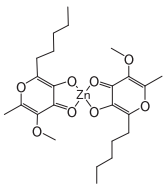
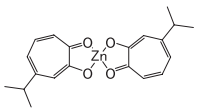
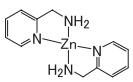
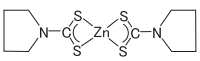
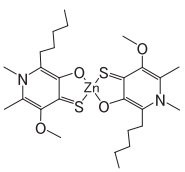
On the other hand, a human trial using vanadyl compound was reported, in which 6-week oral $VOSO_4$ administration at a dose of 150 mg/day improved the hyperglycemic state in type 2 DM patients by reducing basal glucose production and enhancing muscle insulin sensitivity.[33)] Following this observation, the first human trial of $VO(ema)_2$ complex was reported in 2006.[34)]

Other interesting antidiabetic vanadyl complexes with different coordination environments, such as porphyrin complexes, thiazolethione complexes, hydroxydiazine-type complexes, furan complex, quercetin-related complexes, salen-related complexes, and dinuclear complexes, have recently been proposed.[14, 35–37)]

Zinc

Type 2 insulin-resistant DM accounts for 95% of all DM. The therapy for type 2 DM relies mainly on several chemotherapies to reduce hyperglycemia in addition to diet control and exercise. For instance, the therapy includes medicines such as sulphonylureas, which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; thiazo-

Table 6. Typical Antidiabetic Zinc Complexes with Different Coordination Environments[13, 14, 22)]

N_2O_2			
	$Zn(pa)_2$	$Zn(6mpa)_2$	$Zn(6epa)_2$
O_4			
	$Zn(ma)_2$	$Zn(alx)_2$	$Zn(hkt)_2$
N_4			
		$Zn(2ampy)_2$	
S_4			
		$Zn(pdc)_2$	
S_2O_2			
		Zn(tanm)	

lidinediones, which enhance insulin action; and α-glucosidase inhibitors, which interfere with glucose absorption in the small intestine.[38)] Such therapies have limited efficacy, limited tolerability, and some side effects.

To overcome the defects of pharmaceutics for type 2 DM, a new class of oral hypoglycemics, dipeptidyl peptidase-4 (DPP-4) inhibitor, has been approved and used in 2009. The action mechanism of Sitagliptin is thought to result from increased incretin level involving glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP).[39)]

During the struggle to search for new pharmaceutics, several metal ions and their complexes were found to show antidiabetic effect in experimental animals. In particular, studies on Zn were actively conducted. Zn stimulates lipogenesis and glucose transport in adipocytes; its supplementation in diet attenuated hyperglycemia in diabetes (db)/db mice. Clinical research showed evidence for a correlation between Zn deficiency and DM.[40)]

Zn actually exhibits insulinomimetic activity and antidiabetic effects (Table 6). Recently, Zn complexes with excellent antidiabetic and antimetabolic syndrome effects in animals have been found. Cyclo(His-Pro) and arachidonic acid plus Zn, Zn-allixin related complexes, and Zn-dithiocarbamate complexes act as anti-diabetic agents.[13, 14, 28, 41)]

Particularly, bis(thioallixin-*N*-methyl)Zn [$Zn(tanm)_2$][42)] and bis(pyrrolidine-*N*-dithiocarbamato)Zn [$Zn(pdc)_2$][43)] complexes exhibited high hypoglycemic activities on oral administration in KKA^y mice. The OGTT indicated that the blood glucose level attained a peak at 30 min after glucose loading, then reduced gradually, and finally returned to the initial normal level after 120 min in the treated groups; however, it did not recover in the control group. In addition, HbA_{1c} levels improved and serum parameters that are indicative of insulin resistance improved after $Zn(tanm)_2$ and $Zn(pdc)_2$ administration for 25 days. The control KKA^y mice given the vehicle alone exhibited severe hyperinsulinemia, hyperleptinemia, hypertriglyceridemia, and hypoadiponectinemia.

However, the parameters for insulin, leptin, and triglycerid (TG) levels of the KKA^y mice that received $Zn(tanm)_2$ and $Zn(pdc)_2$ were significantly reduced as compared to those of the control KKA^y mice, suggesting that the two complexes improve insulin resistance. Hypoadiponectinemia induced by visceral fat accumulation is known to become a strong risk factor not only for DM and hypertension but also atherosclerosis and cardiac events. Thus, hyposecretion of defensive adipocytokines such as adiponectin might be the major mechanism of lifestyle-related disease, including DM, hyperlipidemia, and hypertension, comprising the metabolic syndrome. The oral administration of $Zn(tanm)_2$ and $Zn(pdc)_2$ complexes may contribute to the treatment of DM and metabolic syndrome in humans.

Action Mechanism

The mode of action of vanadyl compounds has previously been examined by many researchers, and important data have been accumulated with regard to the inhibition of protein tyrosine phosphatase (PTP1B) activity.[44] This activity is involved in the activations of the insulin receptor tyrosine kinase, the cytosolic nonreceptor tyrosine kinase, direct phosphorylation of insulin receptor substrate 1 (IRS1), and the activation of phosphatidylinositol 3 kinase (PI3K), thereby leading to glucose transporter 4 (GLUT4) translocation to the cell membrane. Accordingly, some researches suggest that the suppression of hepatic glucose output through the inhibition of key gluconeogenic enzymes may play an important role in mediating the glucoregulatory effects of vanadyl compounds. In a recent study, the molecular mechanism of $VO(3mpa)_2$, $VO(alx)_2$, and [meso-tetrakis(4-sulfonato-phenyl)porphyrinato]oxovanadium(IV) [VO(tpps)] was revealed to affect the tyrosine phosphorylation of IRβ and IRS, leading to the activation of PI3k-serine/threonine kinase, transforming murine leukemia virus, *akt8* (Akt) signaling and translocation of GLUT4 to the plasma membrane.[13, 14] Furthermore, it was examined whether $VO(alx)_2$ complex contributes to both the activation of the insulin signaling cascade that activates GLUT4 translocation and the regulation of the forkhead box O1 (FOXO1) transcription factor that controls the gene transcription of glucogenesis genes. The critical functions of $VO(alx)_2$ complex have been revealed to involve not only the activation of PI3k-Akt signaling through the enhancement of tyrosine phosphorylation of IRβ and IRS, which in turn transmits the signal to activate GLUT4 translocation, but also the regulation of the DNA binding activity of the FOXO1 transcription factor.[45]

Quite recently, oral administration of $VO(alx)_2$ has been found to restore the impaired activation in signaling cascades related to glucose metabolism and insulin action, and to alter the gene expression in the skeletal muscle of STZ mice.[46]

On the other hand, many studies on the potential protective effect of Zn in the development of DM have been performed in animal models of DM. Zn is a known antioxidant in the immune system. It was tested whether or not an increase in dietary Zn can prevent the onset of type 1 DM by blocking nuclear factor (NF)-κB (one of the gene transcription factors) activation in the pancreas. The results showed that high Zn intake significantly reduced the severity of type 1 DM in alloxan- and STZ-induced diabetic animal models. Zn supplementation also inhibited NF-κB activation and decreased the expression of inducible nitric oxide synthase (iNOS), which is a downstream target gene of NF-κB.[47]

Thus, Zn supplementation was concluded to significantly inhibit the development of type 1 DM. In addition, the effect of dietary Zn deficiency and Zn supplementation on hyperglycemic control in db/db mice was compared. Dietary Zn supplementation attenuated hyperglycemia and hyperinsulinemia in db/db mice. Zn appears to play a role in modulating insulin receptor tyrosine kinase activity in the skeletal muscle of a genetic type 2 DM model mouse.

It was also reported that the effects of Zn on both glucose oxidation and lipolysis stimulation are inhibited by extracellular catalase, which results in H_2O_2 generation. In addition, Zn was observed to stimulate both lipogenesis and glucose transport in adipocytes, and was found to increase the phosphorylation of tyrosine in the β-subunit of the insulin receptor and serine-473 in Akt in adipocytes. Furthermore, it was shown that the enzymatic activity of PTP1B is reversibly inhibited by Zn.[48] These results indicate that Zn affects carbohydrate metabolism through the insulin receptor PTP1B and other related proteins.

In recent years, the action mechanism of Zn complexes has been examined in isolated rat adipocytes or cultured pre-adipocyte fibroblast (3T3-L1) cells, and it has been revealed that Zn complexes, $Zn(pa)_2$ and $Zn(mal)_2$, strongly act on GLUT4 translocation and phosphodiesterase (PDE). Following the study, the molecular mech-

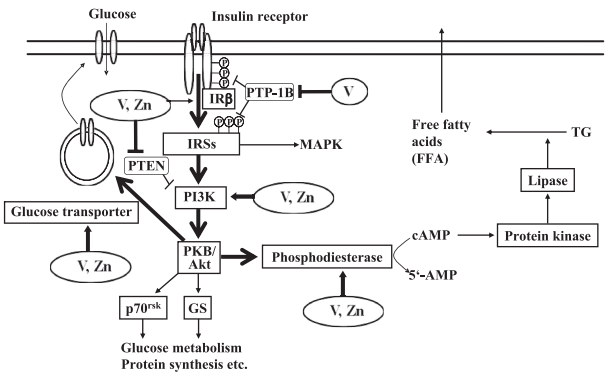


Fig. 11. A Proposed Mechanism for the Antidiabetic V and Zn Complexes[45, 49, 50]

IR is a tyrosine kinase that undergoes autophosphorylation, and catalyses the phosphorylation of cellular protein IRS1. Upon tyrosine phosphorylation, the protein activates PI3K, which in turn activates Akt. Thus, GLUT4 is activated and translocated to the cell membrane, loading to the uptake of glucose into the cell. Potential action sites of vanadyl (VO) and Zn complexes are indicated by two signs (inhibition: ⊣ or activation: →).

anism of Zn complexes was examined and it was found that the complexes exhibit antidiabetic activities by regulating glucose utilization and lipid metabolism.[49, 50]

In conclusion, vanadyl and Zn complexes inhibit PTP1B and activate PI3k-Akt signaling through the enhancement of tyrosine phosphorylation of IRβ and IRS, which in turn transmits the signal to activate GLUT4 translocation (Fig. 11).[45, 46, 50]

ANTIOXIDATIVE METAL COMPLEXES

SOD-mimetic Cu complexes with beneficial ligands have been anticipated to be clinically useful.[51–54] It is well known that Cu^{2+} exhibits $\cdot O_2^-$ scavenging activity, and aspirin (asp) has been used as an antipyretic, an analgesic, and anti-inflammatory drug for many years. Aspirin has also been used as an antiplatelet aggregating, an anticancer, and a protective drug against UV exposure. Subsequently, Cu^{2+}-aspirin [$Cu_2(asp)_4$] complex (Fig. 12) is expected to achieve a synergistic effect. The complex was examined with regard to the ROS suppressive effects in the following events; the chemical $\cdot O_2^-$ scavenging activity, the *in vitro* antioxidative activity in cultured cell systems as human keratinocyte cell line (HaCaT) and normal human dermal fibroblast (NHDF) under an UVB exposure, the effect of oral administration on UVA-

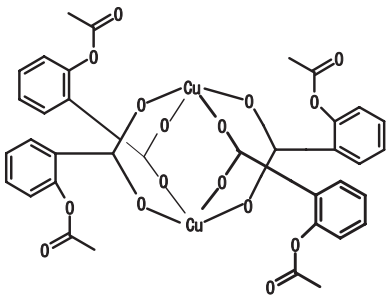


Fig. 12. Structure of Copper(II)-asprinate tetra(asprinato)di-copper(II) Complex[55]

induced ROS generation in hairless mouse skin, Cu distribution in the mice, the metallokinetics of Cu^{2+} in the circulating blood of rats, the metallothionein induction, and SOD levels in the skin of mice. From these results, $Cu_2(asp)_4$ has been proposed to be an orally active antioxidative compound.[55, 56]

It is well recognized that aging-associated nitro-oxidative stress causes tissue injury and activates proinflammatory pathways that play an important role in the pathogenesis of aging-associated cardiac vascular dysfunction. Then the effect of $Cu_2(asp)_4$ complex was investigated, and found that the treatment of the complex in the rats reduces the nitro-oxidative stress and improved cardiac function.[57]

Antioxidative metal complexes with protective ability against skin damage similar to $Cu_2(asp)_4$ complex such as Zn-glycinate complexes have also been proposed.[58, 59] The simple complex has been revealed to suppress UVB-induced melatonin synthesis by stimulating metallothionen expression, which follows the suppression of the oxidative stress.

CONCLUSION

When a compound is developed with the aim of using it in clinical pharmaceutics, the following five points are most important: occurrence of good leading compound, high pharmacological activity, low toxicity and low side effects, good pharmacokinetic (PK) and pharmacodynamic (PD) features in terms of its effective concentration in the blood, and evidence of pharmacological action mechanism. Among these points, the first, second and fifth factors appear to be established in the framework of the researches described in this review. However, research on low toxicity and low side effects and PK and PD features needs to be described in

the future. In fact, approximately 30% of candidate organic compounds have failed to be developed as clinical pharmaceutics owing to the lack of suitable PK and PD features. For determining the toxicity and adverse effects of these complexes, observations of animals for long-term administrations of the target complex over least one or two years are necessary.

Thus, a comprehensive understanding of the above-mentioned five factors in each candidate complex is essential to produce clinically active oral antidiabetic, antimetabolic syndrome and antioxidative complexes in the 21st century.

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