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Synthetic and Biocidal Studies of Zn-Hydrazone Complexes

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ABSTRACT

A series of hydrazone derivatives were synthesized through multi-step reactions. The 2-(2-oxo-1,2,3,4-tetrahydroquinoline-7-yloxy)acetohydrazide was prepared from 7-hydroxy-3,4-dihydroquinolin-2(1H)-one as starting material. Then the condensation of 2-(2-oxo-1,2,3,4-tetrahydroquinoline-7-yloxy)acetohydrazide with different o-hydroxyaldehyde derivatives yielded into hydrazone derivatives . These hydrazone ligands were complexed with Zn (II) yielded complexes . The molecular structures of the hydrazones and Zn (II) complexes were characterized by FTIR, 1H and 13C NMR, LCMS, XRD, DSC-TGA, UV–Visible, Fluorescence and elemental analysis. The conductivity experiments showed that all the complexes are non-electrolytes. This is the first comprehensive review of the biological activity of hydrazone-transition metal complexes. Hydrazone complexes gained much attention because of their antifugial, antibacterial anticonvulsant, and analgesic, anti-inflammatory, antimalarial, antimicrobial, antituberculosis, anticancer, and antiviral activities. Additionally, some of the hydrazone complexes were used in treatment of iron overload diseases. One application, which reflects the importance of hydrazone complexes, is their use in detection and determination of metals and some organic constituents in pharmaceutical formulations.The Zn-hydrazone complexes also have biocidal properties.

KEYWORDS: *biocidal, Zn-hydrazone, complexes, synthesized, constituents, formulations, biological.*

Introduction

A biocide is defined in the European legislation as a chemical substance or microorganism intended to destroy, deter, render harmless, or exert a controlling effect on any harmful organism. The US Environmental Protection Agency (EPA) uses a slightly different definition for biocides as "a diverse group of poisonous substances including preservatives, insecticides, disinfectants, and pesticides used for the control of organisms that are harmful to human or animal health or that cause damage to natural or manufactured products". When compared, the two definitions roughly imply the same, although the US EPA definition includes plant protection products and some veterinary medicines.[1,2]

The terms "biocides" and "pesticides" are regularly interchanged, and often confused with "plant protection products". To clarify this, pesticides include both biocides and plant protection products, where the former refers to substances for non-food and feed purposes and the latter refers to substances for food and feed purposes.

When discussing biocides a distinction should be made between the biocidal active substance and the biocidal product. The biocidal active substances are mostly chemical compounds, but can also be microorganisms (e.g. bacteria). Biocidal products contain one or more biocidal active substances and may contain other non-active co-formulants that ensure the effectiveness as well as the

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desired pH, viscosity, colour, odour, etc. of the final product. Biocidal products are available on the market for use by professional and/or non-professional consumers.[3,4]

Although most of the biocidal active substances have a relative high toxicity, there are also examples of active substances with low toxicity, such as CO_2, which exhibit their biocidal activity only under certain specific conditions such as in closed systems. In such cases, the biocidal product is the combination of the active substance and the device that ensures the intended biocidal activity, i.e. suffocation of rodents by CO_2 in a closed system trap. Another example of biocidal products available to consumers are products impregnated with biocides (also called treated articles), such as clothes and wristbands impregnated with insecticides, socks impregnated with antibacterial substances etc.[5,6]

Biocides are commonly used in medicine, agriculture, forestry, and industry. Biocidal substances and products are also employed as anti-fouling agents or disinfectants under other circumstances: chlorine, for example, is used as a short-life biocide in industrial water treatment but as a disinfectant in swimming pools. Many biocides are synthetic, but there are naturally occurring biocides classified as natural biocides, derived from, e.g., bacteria and plants. Zn-hydrazone complexes are biocides in nature.

The metal complexes of these hydrazones were prepared with Zn(II). All the Compounds were characterized and screened for their biocidal activity against gram + ve (S aureus) and gram - - ve (E. coli) bacteria and two common fungi (*A. niger* and *C. albicans*) by several dilution method in slant and broth culture media. A part from the synthetic studies the chemistry of hydrazones is gaining much importance due to their anti-fungal, insecticidal, tubercular, anti-cancerous, and anti-inflammatory agents. The order of activity infers that the biocidal activity of a synthesized potentially active molecule and its metal complexes is dependent both the nature of metal ions and the nature of the parent acid chosen for the synthesis of hydrazones which make them specific in nature against a particular bacteria or fungi. However the compounds of Zn and Hg have been found more effective biocidal against the chosen bacteria and fungi.[7,8]

New hydrazone ligandsand Zn –hydrazone complexes have been prepared. These ligands and metal complexes were characterized by different spectroscopic techniques. The preliminary results of antituberculosis study showed that the hydrazones exhibited very good antituberculosis activity. Among the tested compound 3b was found to be most active with minimum inhibitory concentration of 1.6 μg/mL against *Mycobacterium tuberculosis*.[9,10]

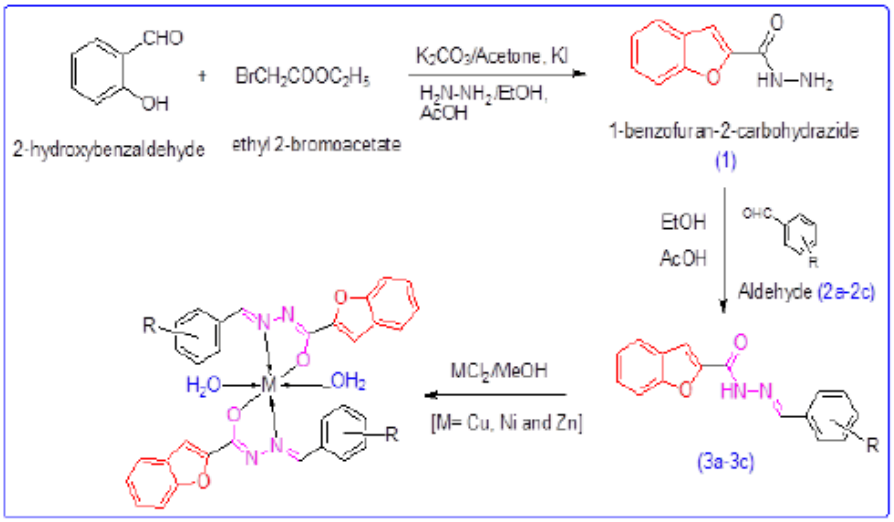

Figure 1: Preparation of hydrazone ligands and metal complexes

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The hydrazone ligand finally refluxed with zinc chloride in ethanol with molar ratio 2:1 to yield zinc complexes. These are insoluble in water but are soluble in DMF and DMSO. They were characterized by different spectroscopic techniques (FT-IR, 1H and 13C NMR, MS, UV–Visible and Fluorescence, TGA and XRD).[13]

The IR spectrum of free ligand shows a broad band at 3316 cm-1, assigned to hydrazone υ(NH) group. The peaks at 1664 and 1608 cm-1 are due to carbonyl υ(C=O) andazomethine υ(C=N), respectively. The peaks attributed to carbonyl υ(C=O) and azomethine υ(C=N) are shifted to lower frequencies in the spectra of all the complexes due to the participation of carbonyloxygen and azomethine nitrogen in coordination. Taken collectively, these spectral data indicate that the hydrazone ligand is behaving as a monobasic bidentate (NO) chelating ligand. The formation of hydrazone is also confirmed by the presence of intense molecular ion peak in the mass spectra of hydrazone derivatives. Spectral evaluation predicts the molecular weights of the desired hydrazone compounds. The first stage involves weight loss below 100ºC due to the removal of the lattice cell water in the complexes. Second step involves weight loss in the temperature range 110°C–160°C is due to elimination of coordinated water. A plateau was observed above 600ºC corresponds to the formation of corresponding metal oxide. While TGA study of Zn (II) do not show any weight loss upto 200°C, indicates absence of coordinated water molecules. The considerable weight loss from 250°C to 450°C indicates loss due to volatile matter. Above 450ºC there is a plateau seen indicates formation of stable zinc oxide(4a)[11,12]

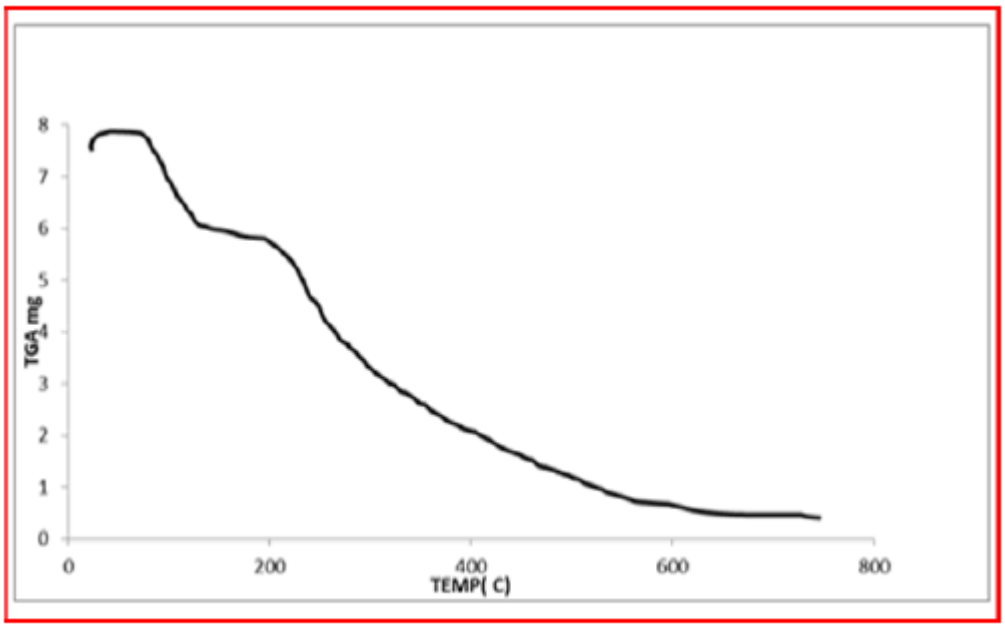

Figure 2: TGA spectra of 4a

Discussion

The ability to selectively and effectively control various molecular processes via specific stimuli is a hallmark of the complexity of biological systems. The development of synthetic structures that can mimic such processes, even on the fundamental level, is one of the main goals of supramolecular chemistry. Having this in mind, there has been a foray of research in the past two decades aimed at developing molecular architectures, whose properties can be modulated using external inputs. [14,15]In most cases, reversible conformational, configurational, or translational motions, as well as bond formation or cleavage reactions have been used in such modulations, which is usually initiated using inputs including, irradiation, metalation, or changes in pH. This research activity has led to the

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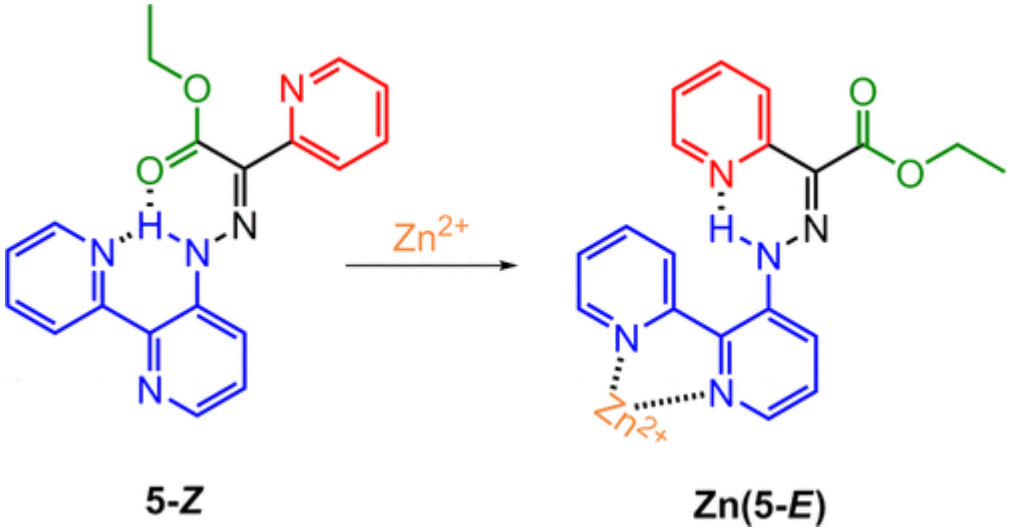
development of a diverse array of impressive adaptive systems that have been used in showcasing the potential of molecular switches and machines. That being said, there are still numerous obstacles to be tackled in the field, ranging from difficulties in getting molecular switches to communicate and work together to complications in integrating and interfacing them with surfaces and bulk materials. Addressing these challenges will necessitate the development of creative new approaches in the field, the improvement of the currently available materials, and the discovery of new molecular switches. Our focus on the modular and tunable hydrazone functional group was instigated by the desire to simplify the structure and design of molecular switches in order to circumvent multistep synthesis. We hypothesized that by avoiding this synthetic bottleneck,[16,17] which is one of the factors that hinder fast progress in the field, we can expedite the development and deployment of our adaptive materials.

The choice of the hydrazone framework as the basis of our molecular switches stems from its modularity, straightforward synthesis, functional diversity, and stability. These properties have enabled the utilization of hydrazones in various fields, ranging from medicinal to supramolecular chemistry. What makes the hydrazone group especially suitable for switching applications is its incorporation of an imine bond that can undergo stimuli responsive E/Z isomerization (i.e., configurational switching). The light induced activation of this process was already known in the literature . when we started working with these molecules. However, its chemical modulation was still uncharted until we showed that it can be accomplished with pH. This dual control over E/Z isomerization is unique to the hydrazone functional group and will be important in our quest to expand the types of intricate motions that we can modulate externally.[18,19]



The straightforward synthesis and functional diversity of the hydrazone functional group enable its use in various stimuli responsive materials. In this Account, we focused on our recent efforts in converting hydrazones into chemically activated molecular switches that can be used in addressing some of the challenges in the field: controlling multistep switching cascades and the photophysical properties of bulk materials. In order to achieve these goals, we had to conduct structure–property analyses that helped us unravel the isomerization mechanism in the intramolecularly H-bonded systems and understand how to control motion around different types of bonds. We also showed that the same systems can be easily converted into two families of fluorophores (i.e., BODIHY and triazolopyridinium dyes), which can be used in sensing applications. Moreover, we demonstrated how coordination to BF_2 can convert the hydrazone switches into visible light activated azo-compounds. These and other applications showcase the multifacetedness of Zn-hydrazones and how this structurally simple group can be used in complicated functions.[20,21]

There are still a myriad of challenges to address before these (and other) molecular switches can be

efficiently used in driving systems out of equilibrium and producing work, among other targeted applications. With the knowledge that we have gained through the past few years, we are now poised to tackle these obstacles. We intend to do this by (i) developing "waste management" strategies (i.e., how to deal with the byproducts of each switching cycle), (ii) controlling cross-talk between different families of switches in order to further complicate the switching cascades, (iii) using the switches in controlling catalytic cycles that can lead to signaling, signal amplification, and feedback loops, (iv) controlling the alignment of the switches in polymers so they can be used as actuators, (v) controlling the timing of the switching events (i.e., temporal control) in order to develop molecular timers, and (vi) using compartmentalization in order to activate parallel and noncompatible switching events (vii) biocidal function. We are confident that these synthetically accessible hydrazones will facilitate and expedite the discovery of complicated switching schemes that when appropriately combined will lead to complexity.

Implications

Due to their intrinsic properties and patterns of use, biocides, such as rodenticides or insecticides, can cause adverse effects in humans, animals and the environment and should therefore be used with the utmost care. For example, the anticoagulants used for rodent control have caused toxicity in non-target species, such as predatory birds, due to their long half-life after ingestion by target species (i.e. rats and mice) and high toxicity to non-target species. Pyrethroids used as insecticides have been shown to cause unwanted effects in the environment, due to their unspecific toxic action, also causing toxic effects in non-target aquatic organisms.

In light of potential adverse effects, and to ensure a harmonised risk assessment and management, the EU regulatory framework for biocides has been established with the objective of ensuring a high level of protection of human and animal health and the environment. To this aim, it is required that risk assessment of biocidal products is carried out before they can be placed on the market. A central element in the risk assessment of the biocidal products are the utilization instructions that defines the dosage, application method and number of applications and thus the exposure of humans and the environment to the biocidal substance.[22,23]

Humans may be exposed to biocidal products in different ways in both occupational and domestic settings. Many biocidal products are intended for industrial sectors or professional uses only, whereas other biocidal products are commonly available for private use by non-professional users. In addition, potential exposure of non-users of biocidal products (i.e. the general public) may occur indirectly via the environment, for example through drinking water, the food chain, as well as through atmospheric and residential exposure. Particular attention should be paid to the exposure of vulnerable sub-populations, such as the elderly, pregnant women, and children. Also pets and other domestic animals can be exposed indirectly following the application of biocidal products. Furthermore, exposure to biocides may vary in terms of route (inhalation, dermal contact, and ingestion) and pathway (food, drinking water, residential, occupational) of exposure, level, frequency and duration.

The environment can be exposed directly due to the outdoor use of biocides or as the result of indoor use followed by release to the sewage system after e.g. wet cleaning of a room in which a biocide is used. Upon this release a biocidal substance can pass a sewage treatment plant (STP) and, based on its physical chemical properties, partition to sewage sludge, which in turn can be used for soil amendments thereby releasing the substance into the soil compartment. Alternatively, the substance can remain in the water phase in the STP and subsequently end up in the water compartment such as surface water etc. Risk assessment for the environment focuses on protecting the environmental compartments (air, water and soil) by performing hazard assessments on key species, which represent the food chain within the specific compartment. Of special concern is a well functioning STP, which is elemental in many removal processes. The large variety in biocidal applications leads

to complicated exposure scenarios that need to reflect the intended use and possible degradation pathways, in order to perform an accurate risk assessment for the environment. Further areas of concern are endocrine disruption, PBT-properties, secondary poisoning, and mixture toxicity.

Biocidal products are often composed of mixtures of one or more active substances together with co-formulants such as stabilisers, preservatives and colouring agents. Since these substances may act together to produce a combination effect, an assessment of the risk from each of these substances alone may underestimate the real risk from the product as a whole. Several concepts are available for predicting the effect of a mixture on the basis of known toxicities and concentrations of the single components. Approaches for mixture toxicity assessments for regulatory purposes typically advocate assumptions of additive effects;. This means that each substance in the mixture is assumed to contribute to a mixture effect in direct proportion to its concentration and potency. In a strict sense, the assumption is thereby that all substances act by the same mode or mechanism of action. Compared to other available assumptions, this concentration addition model (or dose addition model) can be used with commonly available (eco) toxicity data and effect data together with estimates of e.g. LC50, EC50, PNEC, AEL. Furthermore, assumptions of additive effects from any given mixture are generally considered as a more precautionary approach compared to other available predictive concepts.

The potential occurrence of synergistic effects presents a special case, and may occur for example when one substance increases the toxicity of another, e.g. if substance A inhibits the detoxification of substance B. Currently, predictive approaches cannot account for this phenomenon. Gaps in our knowledge of the modes of action of substances as well as circumstances under which such effects may occur (e.g. mixture composition, exposure concentrations, species and endpoints) often hamper predictive approaches. Indications that synergistic effects might occur in a product will warrant either a more precautionary approach, or product testing.chemical[24]

As indicated above, the risk assessment of biocides in EU hinges for a large part by the development of specific emission scenario documents (ESDs) for each product type, which is essential for assessing its exposure of man and the environment. Such ESDs provide detailed scenarios to be used for an initial worse case exposure assessment and for subsequent refinements. ESDs are developed in close collaboration with the OECD Task Force on Biocides and the OECD Exposure Assessment Task Force and are publicly available from websites managed by the Joint Research Centre and OECD

Once a biocidal active substance is allowed onto the list of approved active substances, its specifications become a reference source of that active substance (so called 'reference active substance'). Thus, when an alternative source of that active substance appears (e.g. from a company that have not participated in the Review Programme of active substances) or when a change appears in the manufacturing location and/or manufacturing process of a reference active substance, then a technical equivalence between these different sources needs to be established with regard to the chemical composition and hazard profile. This is to check if the level of hazard posed to health and environment by the active substance from the secondary source is comparable to the initial assessed active substance.

It goes without saying that biocidal products must be used in an appropriate and controlled way. The amount utilized of an active substance should be minimized to that necessary to reach the desired effects thereby reducing the load on the environment and the linked potential adverse effects. In order to define the conditions of use and to ensure that the product fulfils its intended uses, efficacy assessments are carried out as an essential part of the risk assessment. Within the efficacy assessment the target organisms, the effective concentrations, including any thresholds or dependence of the effects on concentrations, the likely concentrations of the active substance used in the products, the mode of action, and the possible occurrence of resistance, cross resistance or tolerance is

evaluated. A product cannot be authorized if the desired effect cannot be reached at a dose without posing unacceptable risks to human health or the environment. Appropriate management strategies needs to be taken to avoid the buildup of (cross)resistance. Last but not least, other fundamental elements are the instructions of use, the risk management measures and the risk communication, which is under responsibility of the EU member states.

While biocides can have severe effects on human health and/or the environment, their benefits should not be overlooked. To provide some examples, without the above-mentioned rodenticides, crops and food stocks might be seriously affected by rodent activity, or diseases like Leptospirosis might be spread more easily, since rodents can be a vector for diseases. It is difficult to imagine hospitals, food industry premises without using disinfectants or using untreated wood for telephone poles. Another example of benefit is the fuel saving of antifouling substances applied to ships to prevent the buildup of biofilm and subsequent fouling organisms on the hulls which increase the drag during navigation.[22,24]

Results and Conclusions

Antibacterial activity of the Zn-hydrazone complexes/ligands was investigated by a previously reported method against different bacterial strains such as *E. Coli, Klebsiella, S. Aureus*, *Enterococci* and fungi species like *Candida albicans* and *Candida krusei*. The nutrient agar medium (Peptone, Beef extract, NaCl and Agar-Agar) and 5 mm diameter paper discs (Whatman No. l) were used. The investigated compounds, i.e. ligands and their complexes, were dissolved (30 µg) in DMF (0.01 mL). The filter paper discs were soaked in solutions of ligands as well as complexes, dried, and then placed in petri plates previously seeded with the test organisms. The plates were incubated for 24 h at 37ºC and the inhibition zone around each disc was measured.

The results of this investigation support the suggested structures of all the Zn-hydrazone complexes. Tetrahedral geometry has been suggested for all the complexes The Schiff base ligands were found to be biologically active and their metal complexes display enhanced antimicrobial activity against one or more strains. Chelation tends to make the ligands act as more powerful and potent bactericidal agents.

Zn-Hydrazone complexes, one of the important classes of organic molecules, are pharmaceutical agents comprising –CO-NH-N=CH- group in the structure therefore and exhibiting significant biocidal activity. Anticancer activity screening results revealed that some compounds showed remarkable cytotoxic effect. These compounds showed high cytotoxic activity against A549 cancer cell line but it showed low cytotoxic effect against normal 3T3 fibroblast cell line. Antiproliferative and antimetastatic effects of these compounds were determined by the real-time monitoring of cell proliferative system (RTCA DP). The cell proliferation, metastatic and invasive activities of cancer cells were decreased due to increased concentration of hydrazone complexes. [24]

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