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IN SEARCH OF SMALL ACTIVE MOLECULES

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Dedicated to

my Father for instilling "Never be afraid to ask why." and
to my Mother for imprinting "And this too shall pass."
These "pedoman" encourage and comfort.

Prologue

My intended purpose in writing this little booklet is to provide the interested reader with an overview of the work which has occupied my collaborators, myself and our graduate and undergraduate students over the last several years.

I hope that those of you who are not as fascinated as we are with the very tiny corner of chemistry described herein will come away with an understanding of why we are driven to continue "in search of small active molecules". I hope that those of you who are interested to further explore this topic will read our publications.

Contents

ABSTRACT

Developments in bioinorganic chemistry, in particular the discovery by Rosenberg in the late 1960s that a simple complex of platinum could be used in the treatment of cancer, led to a resurgence in interest in metal complexes. The search for other small complexes that would be selective, effective and cause fewer side-effects has been intensive over the last several decades.

A series of several S-substituted dithiocarbazates, hundreds of their Schiff bases and their transition and non-transition metal complexes have been synthesized and characterized in our work. Variation of substituent groups on the dithiocarbazato backbone and use of different metals in their complexation produced small compounds with major differences in structure and coordination. Large variations in biological activity and selectivity have been observed even though the changes in the structure and/or orientation of substituent groups have been specifically designed to be minor.

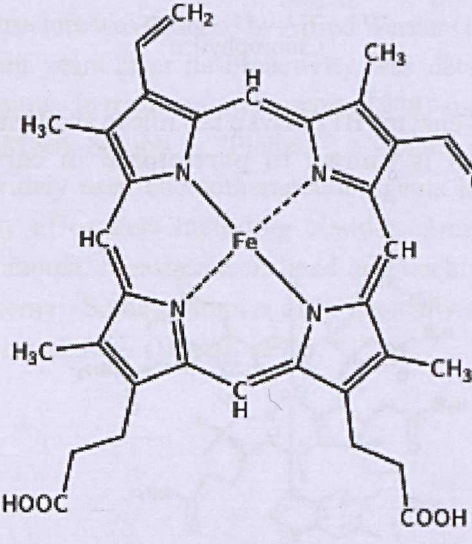
Such small variations enable meaningful application of statistical methods (including QSAR and TSAR, for example) and computational chemistry techniques to the study of the structure-reactivity relationships. A deeper understanding of the action of these compounds gained through these techniques is expected to lead to advances in the design of bioactive compounds.

INTRODUCTION

Bioinorganic Chemistry

Bioinorganic Chemistry is an area of rapid development focusing on the functions of metals in biological systems. Compounds having metal centers – coordination compounds – are important in many biological processes. They are found in proteins, enzymes and co-factors.

Oxygen transport and storage in humans depends upon the iron-porphyrin complex in hemoglobin. Asphyxiation occurs when labile oxygen is replaced by the much more strongly bound hydrogen sulphide, carbon monoxide or cyanide ligands.



Heme Group

INTRODUCTION

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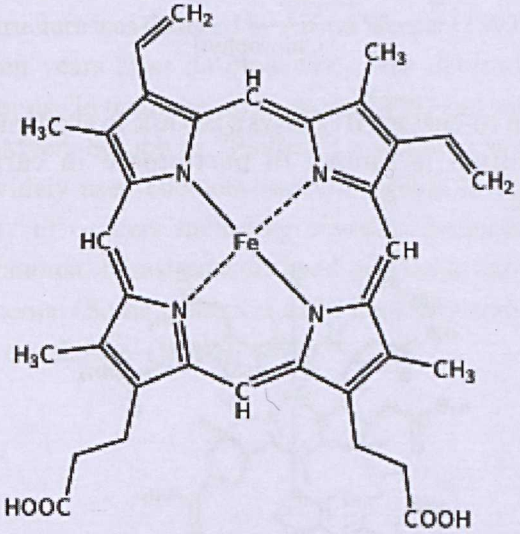
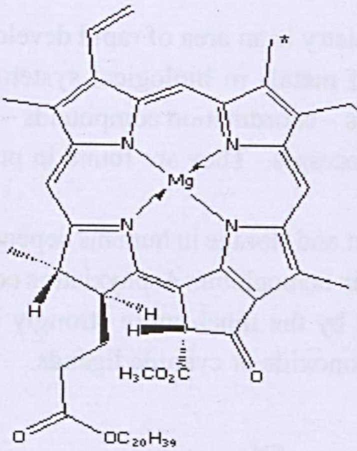


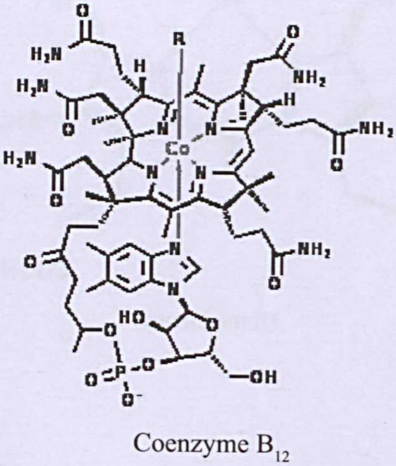
Heme Group

A similar complex containing magnesium in chlorophyll regulates photosynthesis.



Chlorophyll

Cobalt in co-enzyme B12 plays a key role in alkyl transportation and chromium is known to participate in carbohydrate metabolism.



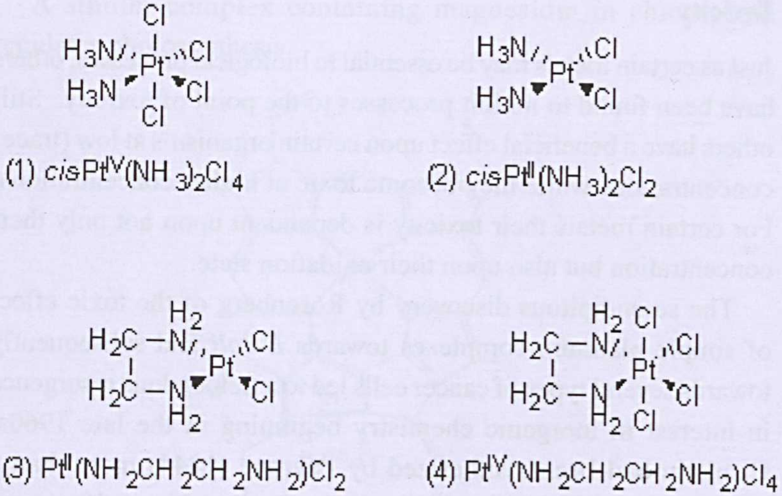
Coenzyme B_{12}

(Jamieson and Lippard,1999)

Toxicity

Just as certain metals may be essential to biological processes, others have been found to inhibit processes to the point of toxicity. Still others have a beneficial effect upon certain organisms at low (trace) concentrations while they become toxic at higher concentrations. For certain metals their toxicity is dependent upon not only their concentration but also upon their oxidation state.

The serendipitous discovery by Rosenberg of the toxic effect of simple platinum complexes towards *E-coli* and subsequently towards several types of cancer cells led to a tremendous resurgence in interest in inorganic chemistry beginning in the late 1960s. Cisplatin had been synthesized by Peyrone (1844) more than a century before Rosenberg's discovery. It was almost fifty years before its structure was deduced by Alfred Werner (1893). However, just over ten years after its bioactivity was determined it was approved for use in treatment in humans (1978) and was marketed by Bristol-Myers Squibb as "Platinol". Cisplatin remains one of the most widely used chemotherapeutic agents in the treatment of a variety of cancers including bladder carcinoma, adrenal cortex carcinoma, breast cancer, head and neck carcinoma and lung carcinoma. Some examples of biologically active platinum complexes are shown. (Bruhn, 1991).


(1) $cis\text{Pt}^{IV}(NH_3)_2Cl_4$

(2) $cis\text{Pt}^{II}(NH_3)_2Cl_2$

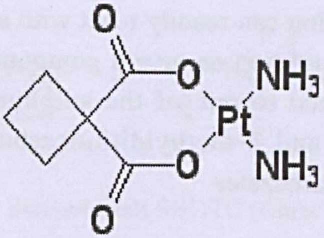
(3) $\text{Pt}^{II}(NH_2CH_2CH_2NH_2)Cl_2$

(4) $\text{Pt}^{IV}(NH_2CH_2CH_2NH_2)Cl_4$

Structures of some biologically active platinum complexes

Unfortunately, most chemotherapeutic agents currently in use are non–selective and attack other types of rapidly dividing cells in the body in addition to the targeted cancer cells. Such cells can be found in the gastrointestinal tract, hair follicles, and bone marrow. For this reason, some of the common adverse side effects of drugs used in cancer chemotherapy are nausea, alopecia (hair loss), and myelosuppression (a reduction of activity in the bone marrow—in particular, toxicity to the blood forming elements).

A derivative of cisplatin, commonly called carboplatin, was developed which exhibits fewer side effects. The structure is given below. It is apparent that this structure does not vary much from that of cisplatin and yet its properties are quite different. Such differences are not uncommon and therefore not unexpected. This has led to a recognition of the need to explore structure-reactivity relationships for biologically active compounds.

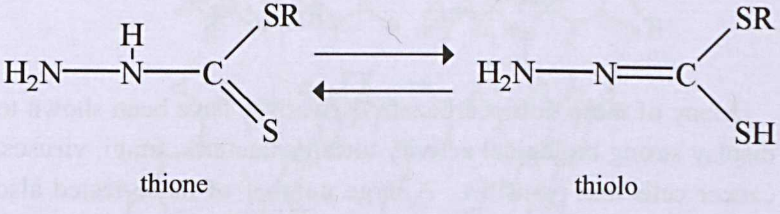


Carboplatin
(Rixe *et al.* 1996)

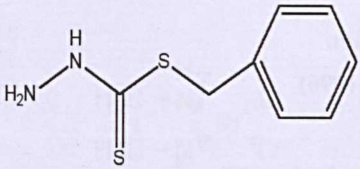
DERIVATIVES OF DITHIOCARBAZIC ACID

Substituted Dithiocarbazic Acid

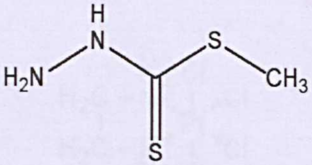
Compounds studied in our laboratory over the last decade have a common source – the dithiocarbazic ion. This anion is produced by the tightly controlled reaction of carbon disulphide and hydrazine in basic ethanolic solution. (Tarafder and Ali, 1978) The ion may occur in two tautomeric forms as shown below. Both tautomers often occur in equilibrium in solution but the ion generally adopts the thione form in the solid state.



Dithiocarbazic ion can readily react with an organic halide by nucleophillic substitution to give a compound with an organic substituent attached to one of the sulphur atoms. S-benzyl dithiocarbazate and S-methyldithiocarbazate are two such substituted dithiocarbazates



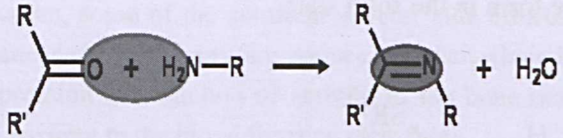
S-benzyldithiocarbazate
SBDTC



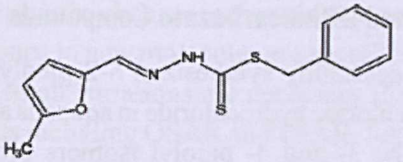
S-methyldithiocarbazate
SMDTC

Schiff Bases

The free amine in the substituted compounds of this group can undergo condensation with an aldehyde or ketone to form an imine – a Schiff base.

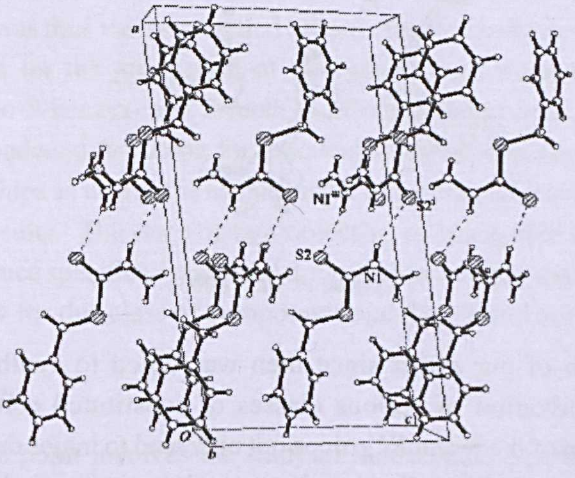


Many of these dithiocarbazato derivatives have been shown to display strong biological activity towards bacteria, fungi, viruses, cancer cells and parasites. A large number of those tested also display antioxidant properties.



A Schiff base derived from SBDTC (Chew *et al.,* 2004)

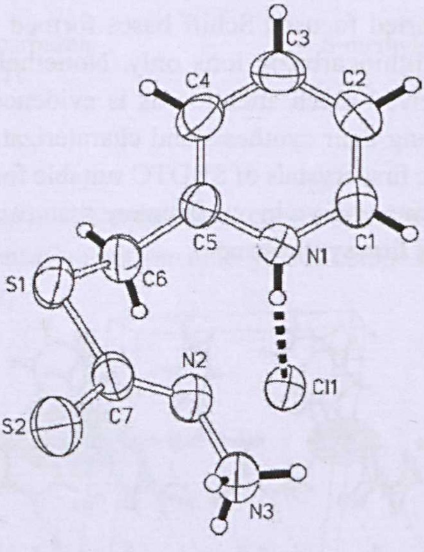
Theoretically, thousands of Schiff bases with the same backbone can be synthesized by varying the organic substituents R and R'. However from 1976 until 2001 with the exception of one report on S-benzoyldithiocarbazoic acid (Tarafder and Bose 1983) all the studies reported focused Schiff bases formed with S-benzyl and S-methyl dithiocarbazic ions only. Nonetheless these two derivatives received much attention as is evidenced by the rich literature detailing their synthesis and charaterization. It should be noted that the first crystals of SBDTC suitable for single crystal diffractometry were grown in our lab more than two decades after the material was first synthesized.



The packing of the molecules of SBDTC viewed down the b axis (Tarafder *et al.,* 2001)

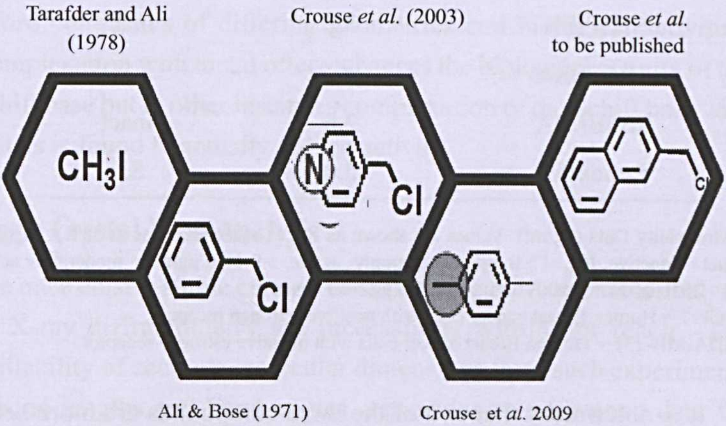
Novel Substituted Dithiocarbazato Compounds

In 2001 we successfully synthesized S-2-picolyl dithiocarbazate using 2-picolylchloride hydrochloride in aqueous alcoholic medium. Surprisingly, the 3- and 4- picolyl isomers proved much more difficult to synthesize and purify. The extended effort necessary to reach this goal was worthwhile, however, since its achievement opened the door to the preparation of an array of other S-substituted compounds and their metal complexes, extensively increasing the number of possible adaptations for compounds in this class.



Crouse *et. al.,* (2003)

Much of our effort since then was tuned to synthesis and characterization of various classes of substituted compounds. Variation of the R and R' groups not only lead to major differences in structure and coordination but to wide variations in biological activity and selectivity. Often large variations in activity are observed even though the changes in the structure and/or orientation of substituent groups in new molecules are specifically designed to be minor. Such small variations are necessary if we are to apply statistical methods including QSAR and TSAR, for example, to the study of the structure-reactivity relationships.



The pool of available small molecules for study of biological activity was thus vastly extended because the S-subsistuted starting materials for the production of the Schiff bases were no longer limited to S-benzyl- and S-methyl-dithiocarbazaic acids. This, in turn, broadened the scope for practical study of structure-activity relationships as well as the advancement of theoretical interpretation of the results. The once distant objective of being able to design and produce specifically targeted drugs is thus brought more clearly into view for this class of compounds and their metal complexes.

Cytoxicity

A case in point involves the study of Schiff bases prepared from halo-substituted isatin (5-fluoro-, 5-chloro- and 5-bromo-isatin)

The data obtained for the bioassays using these Schiff bases against human breast cancer cells is reproduced below. (Accepted for publication in Eur J. Medicinal Chem., 2009)

Sample	**MCF-7**	**MDA-MB-231**
SB5FISA	3.2	inact
SB5ClISA	14.0	inact
SB5BrISA	2.6	inact
Tamoxifen	5.0	8.0

Cytotoxicity Data (μg/ml) Values are shown as IC_{50} (cytotoxic dose at 50%) in μg/ml. inact = inactive, $IC_{50} < 5.0$ μg/ml = Strongly active, 5.0-10.0 μg/ml = moderately active, 10 - 25.0 μg/ml = weakly active, > 25.0 μg/ml = inactive
MCF-7 = Human Breast cancer cells with positive estrogen receptor
MDA-MB-231 = Human Breast cancer cells with negative estrogen receptor

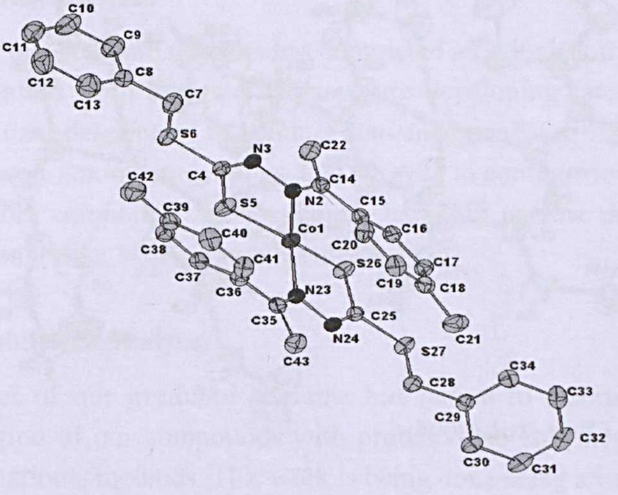
It is noteworthy that two of the three compounds are more active against cells with the positive estrogen receptors than the third. These two are also far more toxic towards the cancer cells than the standard drug used, tamoxifen. All three compounds however are inactive against the breast cancer cells with negative estrogen receptors, unlike tamoxifen, which is active against these cells as well although less aso compared with the positive estrogen receptors. Such differences need to be investigated. The results of such studies may very well prove valuable in developing a greater understanding of these cancers and their treatment.

Metal Complexes

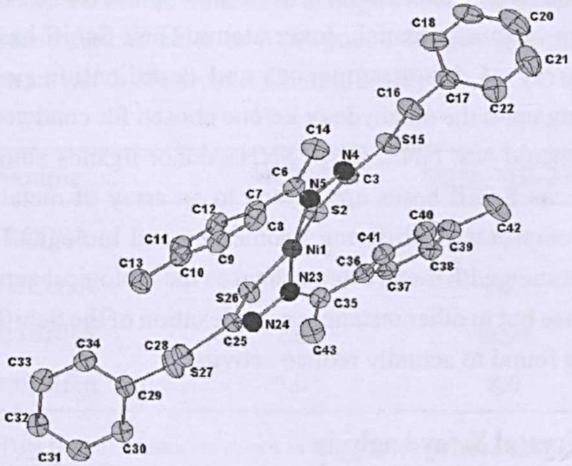
The substituted dithiocarbazic derivatives, SRDTC, and their Schiff bases, though interesting in themselves, become even more intriguing when they are combined with metals. SRDTC ligands where R is the organic substituent sourced from the organic halide, RX, have a minimum of four potential donor atoms. Their Schiff bases display a vast array of donor sequences and coordination possibilities depending upon the aldehyde or ketone chosen for condensation. We have prepared NS, NNS, ONS, SNNS donor ligands among others. When these Schiff bases are ligated to an array of metal ions they afford complexes of differing geometries and biological activities. Complexation with metal often enhances the biological activity of the Schiff base but in other instances complexation of the Schiff base with metals is found to actually reduce activity.

Single Crystal X-ray Analysis

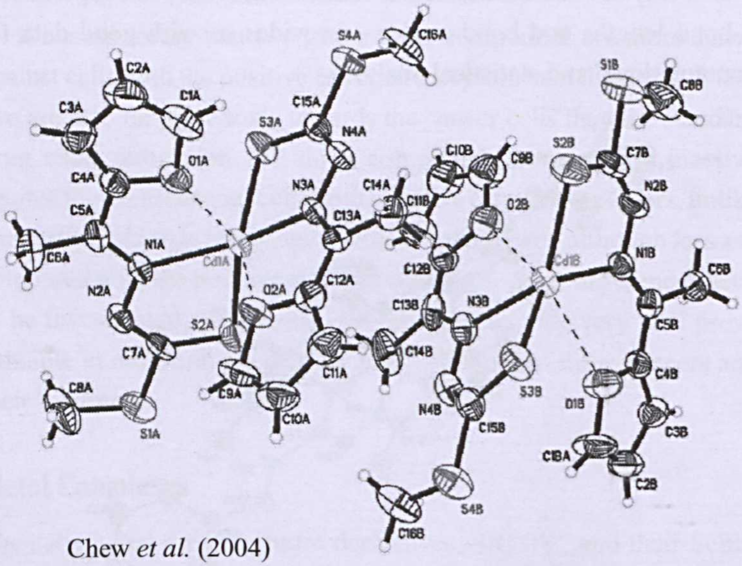
The once elusive single crystals of sufficient quality to enable analysis by X-ray diffractometry are increasingly within our reach. The availability of accurate molecular dimensions from such experiments – bond lengths and bond angles – provides us with good data for computational and statistical analysis.



Co(SB4MA) - Ravoof *et al.* (2007)



Ni(SB4MA)$_2$ – Ravoof *et al. (to be published)*



Chew *et al.* (2004)

BEYOND BIOASSAYS

Mechanistic Studies

To derive meaning from our work beyond academic interest and satisfaction demands that we go beyond bio assays and delve into the action of these compounds upon normal cells as well as diseased cells. It is necessary that the mechanism(s) inviolved at the cellular level be understood. One such study exploring this aspect using gene expression on glioma cell lines U-87M and T98 and normal human brain cells, done in collaboration with Dr Rozita of UPM Medical Faculty and Dr Peter Pok of IMU, has provided us with very encouraging information. Brain cancer is one of the most difficult cancers to treat for many reasons. One metal complex produced in our laboratory has shown remarkable selectivity towards brain cancer with a toxicity towards normal cells that is relatively much lower than tamoxifen.

Statistical Analyses

One of our graduate students has completed an initial study using information from the data bank we are developing to identify operational descriptors to conduct statistical analyses. We have discovered, among other things, that we need to continue to collect data from compounds we synthesize having a narrow range of variation. (How, N.-F., unpublished data)

Computational Analyses

Another of our graduate students has begun to examine the interaction of our compounds with proteins and enzymes using computational methods. This work is being done using an entirely new class of Schiff bases and their metal compounds derived from optically active ketones with an exocyclic carbonyl functionality. Once again many of these compounds have been shown to have very low LC_{50} values. We also observe high selectivity for many of these compounds and their metal complexes as wel as wide variation in thes characteristics with small changes in structural parameters. (Tan, S.L., unpublished data).

CONCLUSION

The study of substituted dithiocarbazates, their Schiff bases and metal complexes have provided us with a wealth of scientific information that has the potential to be put to good use. It has certainly provided our group with much intellectual stimulation and lots of fun!

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BIOGRAPHY

Karen Ann Crouse was born in Sydney, Nova Scotia, Canada. She has been a lecturer the Chemistry Department, UPM since 1972.

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"To Him belongs the Command and to Him will ye (all) be brought back." (28, 88)

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"Every single thing is with Him in due proportion" (13, 8)

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