Review Article

Advances in Bioengineering & Biomedical Science Research

Transition Metal Complexes/Organometallic Compounds as Anticancer/Anti HIV Drugs

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Inorganic chemotherapy has been considerably boosted in recent years by the discovery and exploitation of antitumor compounds based on platinum. Some Gold(III) complexes display similar chemistry. Pharmacological activity to cis-platin and its analogues are now the basis of multimillion dollars industry and bring benefit to thousands of cancer patients. Platinum based anticancer drugs induce normal tissue toxicity particularly to the kidneys and thus alternative metal compounds are presently being evaluated in clinical trials.

General Biological Uses for Metal Containing Drugs

1. Au, Arthritis, cancer
2. Ag , Antisceptic agent
3. Ga, antitumor agent
4. Pt, Pd, Antitumor agent
5. Ru, Rh and Os, Antitumor agents

Many metals are waiting in the wings for biomedical applications including organotin polymers and platinum polymers. Many more may emerge in the near future as signaled by the activity of the small molecules containing metals as drugs.

Anti-Tumor Applications of Transition Metal Complexes

Platinum complexes are applied in half of all chemotherapeutics chemesainst a wide range of tumors. The best known drug is cis-platin.

The design and testing of gold complexes for antitumor activity over the past several decades is based on two rationales: Analogies between square planar complexes of Pt(II) and Gold(III) both of which are d8 ions. Complexation of gold(I) and gold(III) with known antitumor agents to form new compounds with enhanced activity.

Some of the Gold Complexes Tested for Anticancer Activities are

1. Auranofin
2. Au(N-Methylimidazole)Cl3
3. Au(2-Methylbenzoxazole)Cl3
4. Au(2,5-dimethylbenzoxazole)Cl3
5. Au(damp)cl2
6. Au(damp)(SCN)2
7. Au(damp)(OAc)2

Specific Aims

Synthesis and Characterization of Gold and Other Transition Metal Complexes - Anti Cancer Activity Studies

Inorganic chemotherapy has been considerably boosted in recent years by the discovery and exploitation of anti-tumor compounds based on platinum. The active entities are complexes of platinum(ll) which, after metabolism, bind to guanine residues of DNA and inhibit the catastrophic replication characteristic of tumor growth. Gold(lll) complexes display similar chemistry and pharmacological activity to cisplatin; it is now intended to explore a greater variety of gold complexes. My personal views are in (a) expanding this area of co-ordination chemistry, (b) obtaining and screening materials with enhanced biological activity and (c) examining the organ distribution following administration of the compounds. The results would certainly be of potential essential scientific, medical and commercial significance.

Cisplatin and its analogues are now the basis of a multimillion-dollar industry and bring benefit to thousands of cancer patients; gold compounds may prove equally useful. Gold Analogues of Cisplatin the results are of potential scientific, medical and commercial significance. Cisplatin and its analogues are now the basis of a multimillion-dollar industry and bring benefit to thousands of cancer patients; gold compounds may prove equally useful. The compounds mentioned above are the first gold(lll) complexes to have been rigorously shown to have substantial and useful biological activity. It is therefore necessary to explore the rational synthesis of additional materials of this type, to screen their pharmacological properties and to investigate the relevant chemistry and biochemistry.

Hence several complexes of Gold, Platinum, Palladium and Ruthenium would be synthesized from various schiff's bases of Substituted Isatin, Substituted Indoles, Chromones and substituted chromones and other extremely novel ligands and their Anticancer and Anti-HIV studies would be performed. New anticancer drugs would be discovered and if some very important drugs result, they would be patented. Synthesis and Biological Evolution of Novel Analogues of Flavonoids and Their Metal complexes: Mechanism of Induced apoptosis in Human Cancer Cell Lines: Pancreatic, Breast and Prostate Cancer.

The Main Objectives and Aims of the Proposed Work are

Synthesizing analogues of flavonoids, polyphenols vizgenestein, curcumin, resveratrol, flavopiridol and dim: 3-3-diindolylmethane, docetaxel, drugs like gemcitabine etc and other naturally occurring compounds of extreme interest.

Study their biological properties employing highly advanced techniques.

Combining the flavonoids, quercetin, genestein, curcumin, gemcitabin, reservatrol, DIM as precursors with the above mentioned ligands like Diamines, Hydrazones, thiosemicarbazones with our structure based focused approach leading to the identification of bioactive pharmacophores appended to the specially interesting and important moieties involved. These are capable of metal conjugation with especially copper, gold, platinum, ruthenium, zinc and cadmium with potent and enhanced antitumor activity more than the parent compounds.

The Studies Proposed are

Chemical

synthesis and characterization of some organometallic compounds using advanced spectroscopic techniques like Multinuclei NMR, FT-IR, UV-Vis, ESR, Raman, Thermal, Electrochemical, Modelling and Magnetic and X-Ray diffraction studies.

Biological

Anticancer studies, Cytotoxicity, Apoiptosis, DNA binding studies, Structure Activity relationship studies and other Cancer biology studies and assays.

Au(III) Isatin Thiosemicarbazone Complexes

Gold is one of the most promising metals and its compounds are regarded as promising alternatives in cancer therapy and offer many approaches to innovative Metallopharmaceuticals. Thiosemicarbazones are a class of compounds whose biological activity is enhanced by the functional groups of the parent aldehyde or ketone [1]. In cancer treatment metal chelates are more potent than the chelating agents [2]. The Isatin molecule (1H-indole-2, 3-Dione) is a versatile moiety that displays diverse biological activities, including anticancer activity. N-alkylated indoles have also been reported to exhibit anticancer activity indoles derivative vincristine and vinblastine is mainly useful for treating Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, advanced testicular cancer, advanced breast cancer. The derivatives of Isatin-3-thiosemicarbazones exhibit a broad spectrum of biological activity. A full range of gold compounds have been investigated for their potential as anti-tumor agents toward several human tumor cell lines and evaluated in vitro using a systematic screening strategy for their utilization in cancer therapy. The structural features from the spectroscopic and other studies qualify Gold(III) compounds as a promising class of cytotoxic agents of outstanding interest or cancer treatment.

Synthesis of Ligands

Isatin thiosemicarbazones and substituted Isatin Thiosemicarbazones were synthesized by condensing equimolar solutions (0.01M) of Isatin and thiosemicarbazide/4-substituted thiosemicarbazides in methanol in the presence of few drops of acetic acid. The solution after mixing was refluxed on water bath for 2hrs, filtered and the filtrate was concentrated to half the volume. Crystals were obtained on cooling the solution. The crystals were separated and recrystallized from Ethanol.

Synthesis of Metal Complexes

A Solution of Au cl3 was added to Ligands (10^{-3} mole) in ethanol and the reaction mixture was refluxed for 3 hrs. The resulting coloured precipitate was washed several times with methanol and the resulting precipitate was dried in a desiccator over anhydrous $CaCl_2$.

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A Solution of Au cl3 was added to Ligands (10^{-3} mole) in ethanol and the reaction mixture was refluxed for 3 hrs. The resulting coloured precipitate was washed several times with methanol and the resulting precipitate was dried in a desiccator over anhydrous $CaCl_2$. In case of all complexes, the spectra reveal that the bond having a maximum at 1740 cm^{-1} in the free ligand is shifted to lower wave number, the shift being 26-35cm^{-1}. This shift indicates that the carbonyl oxygen atom of the Isatin residue is one of the coordinating sites. The band due to vNH vibration mode in Isatin having maximum at 3190cm^{-1} in the free ligand, remains largely unaffected in the chelates indicating thereby the non-participation of this group in coordination. This shifting of v(c=s) 874 cm^{-1} towards lower side by v 20-30cm^{-1} suggests the involvement of sulphur in coordination. We can therefore conclude that the ligands act as bidentate, the coordination occurring through v(C = S) and v(C=0).

HNMR Spectra

Supplementary data have been obtained by 1H and ^{13}C NMR Spectra which were recorded for the ligands and their Au(III) complexes. HNMR Spectra of the ligand can be resolved into three distinct regions. The spectra exhibit two multiplets at 6.94-7.68 and 6.3-6.8 pm due to the Isatin and amine aromatic rings respectively and one singlet at 10.3 ppm due to the Isatin NH residue. The only 1H.N.M.R signal displaying a downfield shift in complex compounds (from 10.3 to 11.24 pm) are those associated with the hydrogen of the Isatin NH residue. This behavior is related with a decrease of the election density and deshielding of the NH Proton as a result of the participation of the adjacent carbonyl oxygen in coordination [3,4]. This behaviour is in good agreement of the >C = 0 vibration mode appear at regular with the corresponding free ligands. From above discussion it is proposed that all Au(III) TSC complexes are square planner in geometry and the structure of the complexes have been proposed.

Antiproliferative Effects

In vitro cytotoxicity assays were performed to get an insight into the antitumor activity of the novel gold complexes. All complexes as well as the established antitumor drug cisplatin were screened against MCF-7 and MDA-MB 231 breast cancer cells according to established procedures [1,5,6]. IC_{50} values determined after 72 h incubation.

Cisplatin characteristically reduced the cell growth of MCF-7 (IC_{50}= 1.67 μM) and MDA-MB 231 (IC_{50} = 3.95 μM) cells. The lead compound ITAu was distinctly less active than cisplatin at the MCF-7 cell line (IC_{50} = 5.98 μM), while it was 3 fold more active against MDA-MB-231 cells (IC_{50} = 1.33 μM). The cytotoxicity of the isatin gold complexes depended on the substituent at the thiosemicarbazone moiety. An N-methyl group increased the activity against MCF-7 cells (IC_{50} = 1.38 μM) but did not change activity at the MDA-MB-231 cell line. Interestingly, N-ethyl or

N-isopropyl chains reduced the growth inhibitory effects only at MDA-MB-231 cells (IC_{50} = 2.46 and 2.31 µM, respectively). However, both compounds are still more effective than cisplatin. The best results were achieved with the benzyl derivative. IBTAu caused growth inhibition of MCF-7 and MDA-MB-213 cells at nanomolar centrations (IC_{50} = 0.79 and 0.93 µM). Exchange of the benzyl residue by p-toluolyl moiety (IPTAu) strongly reduced the cytotoxic potency to IC_{50} = 2.48 and 4.45 µM, respectively. It is to mention that IcyheAu with an N-cyclohexyl moiety (IC_{50} = 1.10 and 1.22 µM) was only marginally less active than IBTAu. These data clearly document that the in vitro antitumor potency and the terminal N-substituents determine the tumor selectivity. Cellular gold levels in MCF-7 and MDA-MB-231 tumor cells after exposure to 10 µM of the complexes for 4 h (*n* = 3). Nuclear gold levels in MCF-7 and MDA-MB-231 tumor cells after exposure to 10 µM of the complexes for 4 h (*n* = 3). ITAu and IBTAu differ in the cellular uptake. The gold content in MCF-7 cells incubated with IBTAu was 3 fold higher compared to those incubated with ITAu (IBTAu, 132.5 ng/mg; ITAu, 36.00 ng/mg).

Interestingly, IBTAu was 2-fold higher accumulated in MDA-MB-231 (264.9 ng/mg) than in MCF-7 cells (132.5 ng/mg), while the gold content reached with ITAu was nearly the same in both cell lines (36.00 and 42.72 ng/mg, respectively). This means that IBTAu showed an accumulation grade of 7.6 in MCF-7 and 14.6 in MDA-MB-231 cells if the individual cellular parameters were taken into account [2]. To get information if the complexes might reach the major target of metal complexes, the DNA, the nuclei of MCF-7 and MDA-MB 231 cells treated with IBTAu and ITAu or cisplatin were isolated by a short sucrose gradient and investigated for their gold content using ETAAS. The results were calculated as ng of Au/mg of nuclear protein. Cisplatin caused only a low metal content in the nuclei nearly independent on the used cells (MCF-7: 10.00 ng/mg, MDA-MB 231: 7.95 ng/mg). ITAu and IBTAu differ in the cellular uptake. The gold content in MCF-7 cells incubated with IBTAu was 3 fold higher compared to those incubated with ITAu (IBTAu, 132.5 ng/mg; ITAu, 36.00 ng/mg). Interestingly, IBTAu was 2-fold higher accumulated in MDA-MB-231 (264.9 ng/mg) than in MCF-7 cells (132.5 ng/mg), while the gold content reached with ITAu was nearly the same in both cell lines (36.00 and 42.72 ng/mg, respectively). His means that IBTAu showed an accumulation grade of 7.6 in MCF-7 and 14.6 in MDA-MB-231 cells if the individual cellular parameters were taken into account [2]. In the nuclei of cells treated with IBTAu a 50-60 fold higher metal concentration was detected (MCF-7: 643.96 ng/mg, MDA-MB 231: 429.57 ng/mg). Cells treated with ITAu showed a gold level of 127.68 ng/mg (MCF-7) and 136.49 ng/mg (MDA-MB 231), respectively.

This finding points to DNA targeting with gold complexes bearing an isatin ligand. Furthermore, it is very likely that the substituents at the terminal amino group determine cellular and nuclear uptake. If the DNA binding related to the mode of action of the gold complexes is still unclear, because the nuclear metal contents do not correlate with the growth inhibitory effects. The marked "soft" character of the gold (I) center makes on the one hand a selective and tight reaction with the nitrogen donors of nucleobases rather unlikely. On the other hand, recent studies revealing strong interactions with specific protein side chains such as thiols and selenols indirectly support this assumption [3,4]. However, to make a final statement on the mode of action further investigations are necessary.

To get information if the complexes might reach the major target of metal complexes, the DNA, the nuclei of MCF-7 and MDA-MB 231 cells treated with IBTAu and ITAu or cisplatin were isolated by a short sucrose gradient and investigated for their gold content using ETAAS. The results were calculated as ng of Au/mg of nuclear protein. Cisplatin caused only a low metal content in the nuclei nearly independent on the used cells (MCF-7: 10.00 ng/mg, MDA-MB 231: 7.95 ng/mg).

Experimental Part Cytotoxicity

The human MCF-7, MDA-MB 231 breast and HT-29 colon cancer cell lines were obtained from the American Type Culture Collection. All cell lines were maintained as a monolayer culture in L-glutamine containing Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose (PAA Laboratories, Austria), supplemented with 5% fetal bovine serum (FBS; Biochrom, Germany) in a humidified atmosphere (5% CO_2) at 37°C. Calculated as mean of at least two or three independent experiments.

Atomic Absorption Spectroscopy (AAS)

ETAAS measurements were performed according to a previously published standard addition procedure with some minor modifications.1In short: to 100 µL aliquots of the diluted lysates increasing amounts of aqueous gold standard solutions were added. All probes were adjusted to a final volume of 200 µL using twice distilled water, each 20 µL triton X-100 (1%) and ascorbic acid (1%) were added and the probes were measured as described below. The gold content of the lysates was accessed by the linear extrapolation method. A Vario 6 electrothermal atomic absorption spectrometer (Analytic Jena AG) was used for the gold measurements. Gold was detected at a wavelength of 242.8 nm with a band pass of 0.8 nm. A deuterium lamp was used for background correction. Probes were injected at a volume of 25 µL into regular graphite wall tubes. Drying, atomization and tube cleaning steps were performed as outlined in more detail in the literature 1. The temperature for pyrolysis was set to 1200°C. The mean AUC (area under curve) absorptions of duplicate injections were used throughout the study. The limit of gold detection using biological samples as described above was 1.7 µg/L.

Brief Introduction of Various Studies and Systems

Cancer: Cancer is an extremely deadly disease. It is an undoubtedly one of the main health concerns facing our society and one of the primary target regarding medicinal chemistry. Even though platinum based complexes had been in primary use on research on chemotherapy agents, the interest in this field has shifted to non-platinum based agents, in order to find different metal complexes with less side effects and similar or better cytotoxicity. Thus a variety of compels with gold are being studied with non-platinum based ligands, especially gold and Ruthenium and these could someday become the replacement for platinum based anti-cancer drug.

Thiosemicarbazones

One of the most promising areas in which thiosemicarbazone compounds are being developed is their use against cancer. Their anti-tumour activity is extremely differentiated and it is very much dependent on the typology of tumour cells. Large number of thiosemicarbazones has been evaluated for their antitumor activity, because of their useful chemotherapeutic properties. In cancer treatment it has been shown that metal chelate are potent than the chelating agent. Metal complexes based on gold are being intensively

studied as platinum replacements [7-13]. There is every need to develop these gold complexes as they are iso-electronics. The redox properties of the quercetin derivatives has been found to block cell cycle progression in a variety of malignant cell lines including those derived from the prostrate, brain, breast, pancreas and colon. Quercetin derivatives vincristine and vinblasine is mainly useful for treating Hodgkin's disease, lymphocytic lymphoma, advanced testicular cancer and advanced breast cancer. Scientists of the Henan University, China, concluded that quercetin could improve therapeutic index of doxorubicin, a drug used in cancer chemotherapy.

Synthesis and Characterization of Biologically Important Novel Quercetin Thiosemicarbazone Derivatives of Pd(II) Complexes

A molecule of quercetin contains five hydroxyl groups whose presence determines the compounds biological activity and the possible number of derivatives. Quercetin 3-0-glycosides occur as mono saccharide's with glucose, galactose. This quercetin 3-0-glycoside was found in mango fruits. Quercetin may contain up to five ether groups in various configuration. Ether derivatives of quercetin, which also contain sugar substituent.

Synthesis of ligands

A $1x10^{-3}$ M solution prepared by dissolving appropriate amounts thiosemicarbazide in 50ml methanol and 2ml of glycial acetic acid was added drop wise to a $5x10^{-2}$ M solution of quercetin in 50ml methanol while stirring and refluxed for 2-3 hours the product that separated was recrystallized was based on elemental analysis, via, FT-IR HNMR, C NMR, ESR, U.V methods.

Conclusion

Absorption and metabolism of quercetin and its derivatives has attracted much attention in relation to their pro-healthy value. The total flavonoid in take from dietary sources is estimated to be from several hundred milligrams to 1g per day. Quercetin derivatives, glycosides in particular, represent a considerable part of these food constituents. It is common knowledge that having been ingested both quercetin derivatives undergo many metabolic conversions and appear in body tissues. An investigation on the bio availability and metabolism of quercetin derivatives focused mostly on glycosides which was predominates in diet.

Synthesis of Metal Complexes

To 30ml of Pd(II) solution ($5x10^{-2}$M) in methanol was added ($1x10^{2}$M) QTSC in methanol and the mixture refluxed for about 1hour in a separate reflux arrangement. The solid that separated was filtered and washed with water and recrystallized with methanol.

General Procedure for Substituted Thiosemicarbazide and Synthesis, Characterization of Quercetin Thiosemicarbazone Metal Complexes

0.2 Mole of thiosemicarbazide was dissolved in a mixture of 30ml of water and 20g of concentrate sulphuric acid. 0.4 mole of corresponding amine was added and further 90g of sulphuric acid was then added drop wise at 500c cooled and poured in to 300g of ice. It was twice extracted with chloroform and the aqueous layer was then rendered alkaline by adding sodium hydroxide solution. The crystals that precipitated were filtered off, washed with water and dried methanol/ethanol. Thiosemcarbazide of M.P.1900c (from ethanol) was obtained. The yield was 75%. All the other substituted thiosemicarbazides were prepared using the same procedure with the corresponding amine.

The H-NMR δ (CD3OD), 6.18(1H, d, J=1.7Hz), 6.40(1H, d, J=1.7Hz) are due to meta-coupled protons of A-ring (H-6 and H-8) of a flavonoid nucleus. Signals at δ = 6.89 d =8.3Hz, 7.68d, 2.5Hz and δ = 7.55dd, 2.2Hz, 8.3Hz are assigned to H-5', H-2' and H-6' of the ring. 13C-NMR spectra analysis 15, carbon signals typical of flavonoid nucleus. Two protons of the NH_2 group 11.179ppm (S, 1-H, -NH). 11.824ppm (S, 1-H, - NH). These suggest a thiosemicarbozone nucleus. 13C-NMR spectral data indicate in all the complexes downfield or up field chemical shifts are obtained for the carbon resonances adjacent to the assumed coordination sites while the others remain essentially unchanged. The affected carbon resonances in the ligands are shifted or absent, supports coordination. All the metal complexes are stable at room temperature non hygroscopic, sparingly soluble in methanol or ethanol and fairly soluble in DMSO. The analytical data for ligand and metal chelates are consistent with their proposed molecular formulae. The signal at 11.90ppm in the spectrum of ligand due to 2, NH is present in most of the complexes with down field shifts (11.40, 11.43, 11.63) and with an up field shift for one complex (12.41) probably indicating a change in the nature of NH resonance complexes. The signal at 11.90ppm in the spectrum of ligand due to 2, NH is present in most of the complexes with down field shifts (11.40, 11.43, 11.63) and with an up field shift for one complex (12.41) probably indicating a change in the nature of NH resonance.

The 1H NMR spectrum showed protons at aromatic regions from 6-8ppm, and strong hydrogen bonding at 12.5ppm. These suggest a quercetin nucleus. The NMR spectrum of metal chelates confirms the non-participation of NH_2 group in the coordination with metal ions. 1H NMR signals are well defined and the spectrum of ligand exhibits two resonances for the NH_2 protons at 7.8 ppm, a result explained in terms of hindered rotation about the C(S)-4NH_2 bond due to its partial double bond character. The metal complexes show only one resonance due to 4,NH_2 protons, upfield for some complexes (at 9.46, 8.30, 8.23) and down field (at 9.72) for some complexes.

Magnetic Studies

The magnetic moments of metal complexes are found to be subnormal, which may be attributed to the presence of magnetically coupled metal centers in dimeric complexes. Biological Studies: The bioactivity of quercetin derivatives and its impact and human health is still at the developmental stage. Research into the biological properties has found that the flavonoid derivatives are popular as Antioxidants. As a result, quercetin derivatives are utilized as ligands for the complexation with transitional metal. The uniform complexes with highly advance spectroscopic, X-ray diffraction and other techniques and also to test the compounds for their potential use an Anti-cancer, using mammalian cell lines and micemodel. It is common knowledge that metabolic modification of QTSC derivatives alters their antioxidant properties. A human body needs these substances to absorb and use vitamin-C.

We have also found that QTSC Pd(II) complex and rutin contribute to the relaxation of smooth muscles in mammals. Similar properties were observed in methoxyl derivatives of quercetin. Due to its Antioxidant activity, rutin protects liver cells and suppresses hemoglobin oxidation.

Rutin has also Anti-inflammatory properties, which are displayed mostly in respect of chronic diseases. When administered to rats, rutin has also been found to display chemo preventive properties,

acting as an agent blocking carcinogenesis induced by heterocyclic amines.

Biological Studies

The bioactivity of quercetin derivatives and its impact and human health is still at the developmental stage. Research into the biological properties has found that the flavonoid derivatives are popular as Anti-oxidants. As a result, quercetin derivatives are utilized as ligands for the complexation with transitional metal. The uniform complexes with highly advanced spectroscopic, X-ray diffraction and other techniques and also to test the compounds for their potential use an Anti-cancer, using mammalian cell lines and mice model. It is common knowledge that metabolic modification of QTSC derivatives alters their antioxidant properties. We have also found that QTSC Pd(II) complex and rutin contribute to the relaxation of smooth muscles in mammals. Similar properties were observed in methoxyl derivatives of quercetin. Due to its Anti-oxidant activity, rutin protects liver cells and suppresses hemoglobin oxidation. Rutin has also Anti-inflammatory properties, which are displayed mostly in respect of chronic diseases. The Formulae of the complexes could be written as: [ML] n Where M = Pd(II): L=QTSC All complexes show much enhanced nuclease activity in the presence of oxidant, which may be due to free radical reaction (OH*) with DNA.

Nuclease Activity Studies

The production of hydroxyl radicals due to the reaction between H_2O_2 and the metal complexes. The OH* radical involves oxidation of de-oxy ribose moiety followed by hydrolytic cleavage of sugar phosphate backbone. On the basis of physicochemical and spectral data the metal chelates are assigned to be Octahedral complexes. 4.0 Conclusion we have synthesized Ligands of QTSC and their complexes with Pd(II). All complex's plausible structures are supported by IR, NMR, ESR, Electronic spectral data. The ligands and their complexes would be screened for their anti-cancer activity against certain cancer cell lines.

We have developed a simple, convenient and effective method for the synthesis of complexes. To our knowledge, this is the first report of an efficient general method for the synthesis of different Pd(II) complexes of Quercetin Thiosemicarbazone. When administered to rats, rutin has also been found to display chemo preventive properties, acting as an agent blocking carcinogenesis induced by heterocyclic amines. The Formulae of the complexes could be written as: [ML] n: Where M = Pd(II): L=QTSC. All complexes show much enhanced nuclease activity in the presence of oxidant, which may be due to free radical reaction (OH*) with DNA. On the basis of physicochemical and spectral data the metal chelates are assigned to be octahedral complexes.

Synthesis and Structural Determination of Novel Curcumin Oxime Gold(III) Complex: Potential Chemopreventive Drug

Cancer is a deadful disease and any practical solution in combating this disease is of paramount importance to public health. Cancer patients have burdened by drug induced toxic side effects, and no turned to seek help from the complementary and alternative medicine hoping for a better cure. Cancer is a class of diseases characterized by out-of-control cell growth. Cancer harms the body when damaged cells divide into uncontrollably to form lumps or masses of tissue called tumors. Tumors that stay in one sport and demonstrate limited growth are generally considered to be benign. Cancerous cell manages to move throughout the body using the blood systems, destroying healthy tissue in a process called invasion. Cur cumin is a natural polyphenol. It is highly potential molecule capable of preventing and treating various cancers. The anticancer potential of cur cumin is severely affected by its limited systemic and target tissue bioavailability and rapid metabolism.

Various dietary chemo preventive agents, turmeric powder or its extract are broadly used as therapeutic preparations in Indian System of medicine. In the present research work, we provide a summarized synthesis and structural determination of Curcumin Oxime derivative of Gold(III) complex.

The use of these analogs for prevention of cancer tumor progression and treatments of human malignancies. The herbal traditional medicinal plants are important raw material to synthesis the phytochemicals [14,15]. The hytochemical, curcumin is one of the major dietary flavonoid, belonging to a group of flavonol [16]. Flavonoids have a long history of use in traditional medicines in many cultures. Research in recent years has focused on several possible helpful effects of curcumin, including its potential role in preventing cancer. Recent studies suggest that curcumin can slow the growth of cancer cells because they have anti-tumor and anti-oxidant properties [2]. Curcumin and its analogs, we would like to provide a brief survey on phytochemicals in general because phytochemicals are becoming the novel backbone of medicinal chemistry and having broad variety of biologically active compounds produced by plants such as β-carotene, ascorbic acid, folic acid, vitamin E and many others that possess either antioxidant capable of scavenging out certain reactive oxygen radicals or anti-toxicity actions. Several dietary phytochemicals are recommended for prevention of carcinogenesis. Aggarwal, et al. have recently reviewed the cell signaling pathways in cancers that are disrupted by agents isolated from natural origins curcumin.

Curcumin is a simple symmetrical β-diketone and incorporates several functional groups. The two aromatic rings containing phenolic groups are connected by two α, β-unsaturated carbonyl groups. A large number of oximes have been evaluated for their anti-malarial and anti-tumor activities, because of their useful chemotherapeutic properties. In cancer treatment it has been shown that the metal chelates are more potent than the chelating agents. Metal complexes of Gold containing nitrogen and oxygen donor ligands is found to be effective catalysts for oxidation, reduction, hydrolysis and other organic transformation [17-20]. The redox properties of the Curcumin derivatives has been found to block cell cycle progression in a variety of malignant cell lines including those derived from the prostrate, brain, breast, pancreas and colon. A stable crystalline form of Identification of the product was based on elemental analysis viz., FT-IR, HNMR, Mass Spectroscopy, ESR etc.

Synthesis of Metal Complexes

To 30 ml of Gold(III) solution ($5X10^{-2}$M) in methanol was added ($5x10^{-2}$ M) curcumin oxime/curcumin in methanol and 2 ml of glycial acetic acid was added drop wise to the mixture refluxed for about one hour in a separate reflux arrangement. The solid (orange) that separated was filtered and washed with water and recrystallized with methanol.

The compound was characterized using IR, 1HNMR and Mass spectrophotometric techniques. From the analysis the compound was identified.

Curcumin oxime

IR Spectra

IR: 3420.49(labile e), 1635.35(c=o), 1603.62 and 1561.36(aromatic), 1240.60(c=o), 831.20 cm-1 (ligand). HNMR: 1.2 – 1.4(t,3H), 3.2– 3.4(q,2H), 3.9(s,1H), 6.6 – 7.0(m,7H), 7.2 – 7.3(m,2H), 7.4 – 7.5(dd,4H). These suggest the curcumin oxime (ligand) Curcumin gold(III) chloride.

IR

3258.46(labile e), 1597.89 and 1511.63(aromatic), 1223.06(c=o), 1167.09(c=o), 832.01 cm-1 (metal complex). 1 HNMR: 1.2 – 1.4(t,3H), 3.1 – 3.3(q,2H), 6.2 – 7.0(m,5H), 7.0 – 8.0(m,4H).

These suggest the curcumin gold complex. Curcumin oxime gold(III) chloride: IR Spectra: 3416.15(labile e), 1608.02 and 1512.35(aromatic), 1217.49 and 1171.29(c=o), 521.86 cm-1(metal complex). HNMR Spectra: 1.3(t,3H), 3.2(q,2H), 3.5 – 4.0(br,1H), 6.7(s,1H), 6.8(d,5H), 6.8 – 7.0(dd,3H), 7.2 – 7.4(t,3H), 7.5(dd,5H). These suggest the Curcumin Oxime gold complex.

Mass Spectra

276, base peak (removal of 2 –OCH 3 and 2 –OH): 304, by removal of four "O" atoms got this peak and 368, indicates the curcumin. From 11 and 12 on metal complexation both the bands are shifted to lower frequency indicating their involvement in gold complexation. The introduction of oxime functionalities in the curcumin nucleus is best diagnosed from the symmetric and asymmetric bands near the 3416 cm^{-1}.

ESR Spectral Analysis

Electronic Spin Resonance spectra of Gold(III) complex was recorded in DMF at liquid nitrogen temperature value equal to 26.

Magnetic Studies

The magnetic moments of metal complexes were found to be subnormal, which may be attributed to the presence of magnetically coupled metal centres in dimeric complexes.

Biological Activity

The nuclease activity of present ligand and their complexes has been investigated on pBR 322 plasmid DNA by agarose gel electrophoreses in the presence of H_2O_2.

At micro molar concentration, the ligands exhibit no significant activity in absence and in the presence of the oxidant. The nuclease activity was greatly enhanced by incorporation of metal ions and the ligands.

All complexes shows much enhanced nuclease activity in the presence of oxidant, which may be due to free radical reaction with DNA. The interaction of $AuCl_3$ with ligand yields compounds having composition. All complexes are found to be diamagnetic at 312 K indicating essentially a planar geometry for these compounds. The IR spectra of ligand are characterized by the presence of a single strong band at 1635 cm-1 due to the central carbonyl absorptions functionalities which are equivalent. The generation of the oxime functionality at one of the carbonyls can be ascertained from the presence of band at 1561 cm^{-1} and strong carbonyl absorption at 1512 cm^{-1}.

Conclusion

We have synthesized the curcumin/curcumin oxime gold(III) complexes. All complexes plausible structures were supported by LSI mass spectral data along with physico-chemical and IR, HNMR, MASS, ESR spectral data.

The ligands and their complexes would be screened for their anti-cancer activity against certain cancer cell lines. We have developed a simple, convenient and effective method for the synthesis of complexes. To our knowledge, this is the first report of an efficient general method for the synthesis of different gold complexes of curcumin.

Synthesis and Characterization of Novel Curcumin Thiosemicarbozone Gold(III) Complex: Potential Chemopreventive Drug

Curcumin is a natural polyphenol. It is highly potential molecule capable of preventing and treating various cancers. The anticancer potential of cur cumin is severely affected by its limited systemic and target tissue bioavailability and rapid metabolism. Various dietary chemo preventive agents, turmeric powder or its extract are broadly used as therapeutic preparations in Indian System of medicine [21-23]. In the present research work, we provide a summarized synthesis and characterization of Curcumin thiosemicarbozone derivative of Gold(III) complex. The use of these analogs for prevention of cancer tumor progression and treatments of human malignancies.

Synthesis of Metal Complexes

To 30 ml of Gold(III) solution ($5X10^{-2}$ M) in methanol was added ($5x10^{-2}$ M) curcumin thiosemicarbazone in methanol and 2 ml of glycial acetic acid was added drop wise to the mixture refluxed for about one hour in a separate reflux arrangement. The solid (orange) that separated was filtered and washed with water and recrystallized with methanol.

ESR Spectral Analysis

Electronic Spin Resonance (ESR) spectra of Gold(III) complex was recorded in DMF at liquid nitrogen temperature value equal to 30.

Electronic Spectra

The structure of the CurTSC Gold(III) complex is Octahedral. By the recent study of X-ray and polarized single crystal spectral studies of the Au(III) ions, these complexes are diamagnetic. They did not show any bands in the electronic spectra.

Nuclease Activity Studies

The nuclease activity of present ligands and their -complexes has been investigated on pBR 322 plasmid DNA by agarose gel electrophoresis in the presence of H_2O_2. At micro molar concentration, the ligands exhibit no significant activity. The nuclease activity is greatly enhanced by incorporation of metal ions in the ligands.

In absence of oxidants, the Gold(III) Complexes of all CURTSC cause discernible DNA cleavage as evidenced by increase in intensity in form 11 (nicked) and form III (linear) with decrease in intensity in form 1 (super coiled) which is attributed to step-wise conversion of form I to form II and to form III Similar observations were also evident in the Gold(III) Complexes of all CURTSC. The Nuclease Activity of the Gold(III) complexes is more. All complexes show much enhanced nuclease activity in the presence of oxidant, which may be due to free radical reaction (OH*) with DNA. The production

of hydroxyl radicals due to the reaction between H_20_2 and the metal complexes. The OH* radical involves oxidation of deoxyribose moiety followed by hydrolytic cleavage of sugar phosphate backbone [24,25].

Field of Research

This relates generally to novel analogs of isoflavone and metal complexes thereof, and more particularly to isoflavonoid or isoflavonoid mimetics that are useful for preventing and/or treating diseases, such as cancer. Naturalchemoprotective agent. Isoflavones in soy, including genistein, daidzein, glycitein and others, active agents in this regard. Indole-3-carbinol, curcumin, resveratrol and green or black tea polyphenols, genistein is a promising agent for cancer prevention and/or treatment. NF-κB, Akt and MAPK pathways, Sarkar, et al., The Role of Genistein and Synthetic Derivatives of Isoflavone in Cancer Prevention and Therapy, Mini-Reviews in Medicinal Chemistry, genistein causes a pleiotropic effect on cancer cells. However, genistein alone may not be potent enough to treat and/or prevent cancers. There is, therefore, a need for synthetic analogs or derivatives of the isoflavonegenistein that have more robust biological properties. Genistein also exerts its own biological effects that are distinct from estrogen. Since biological activity is related to molecular structure, changes in molecular structure have been shown to cause extensive changes in biological activity.

Isoflavonoidanalogs and their metal complexes as anti-cancer agents. Therefore, derivatives of genistein (and consequently hormone mimetics) based on the structural motifs of genistein and other naturally-derived known phytochemicals, may have greater ability to prevent and/or treat cancers than the natural products themselves. Certainly, synthetic derivatives of these natural phytochemicals maybe easier to produce on a commercial scale. Many of these chemical entities are consumed by humans in nutritional sources which help provide the physiologically active constituents needed for maintenance of life and health. Some of these chemicals are inert, some are toxic or carcinogenic, while others may have positive effects on physiologic function acting as protective agents countering the risk of acute toxicity and diminishing the onset of chronic diseases including cancer. There is, thus, a need for synthetic analogs or derivatives of isoflavone and other naturally-occurring dietary agents that are more potent than the naturally-occurring product and that can be synthesized on a commercial scale.

Isoflavonoid analogs and their metal complexes as anti-cancer agents. There is also a need for a method of rapidly screening the many possible combinations of small molecule analogs of these naturally-occurring compounds for efficacy and toxicity. The foregoing and other objects are addressed by this invention which provides active pharmacologic agents for treating and/or preventing cancer, among other diseases and conditions and particularly breast, prostate and pancreatic cancer, in humans and animals. The active pharmacologic agents of the present invention selectively target receptors of the type over-expressed in malignant cells and comprise ligands of a cytotoxic pharmacophore covalently attached to a carrier. The carrier may be an isoflavonoid or an isoflavonoid mimetic. As used herein, the term "isoflavonoid mimetic" refers to a molecule that has a steroidal motif derived from isoflavone, and in particularly preferred embodiments, from the lavonegenistein.

The isoflavonoid mimetic may be, in some embodiments, a non-fragmented steroidal hormone, such as progesterone or estrogen, or in other embodiments, a small molecule analog of isoflavone, such as 3-formylchromone.

A comparison of the IC_{50} values of present metal conjugates with those of genistein revealed substantial decrease indicating therapeutically achievable efficacy. Since all Gold compounds are redox active metal conjugates, we believe that redox triggered oxidative stress may be one of the underlying mechanisms for the observed apoptotic cell death in the present case. The quantitative evaluation of the apoptosis by ELISA. Referring to the IC_{50} values of these compounds as presented in exhibited the lowest IC_{50} value compared to the other copper conjugates in inhibiting Akt kinase activity. It is interesting to note, however, that while Akt kinase activity could be inhibited with 100 nM (IC_{50}) of FPA-124, the IC_{50} for inhibiting cell growth is 70-100 folds greater than the inhibition of kinase activity. Activation of NF-κB in cancer cells has been shown to attenuate apoptosis induced by chemotherapeutic agents resulting in lower cell-killing and drug resistance. Since the NF-κB pathway is regulated by Akt protein, the effects of the FPA-124 on NF-κB activity was studied in an in vivo experiment, specifically the well-established orthotopic pancreatic tumor model using COLO 357 cells. The parent genistein compound has been shown to inhibit the activity of NF-κB and the growth of hormone dependent (LnCaP) and hormone independent (PC3) human prostate cancer cell lines in vivo without causing systemic toxicity.

Platinum -DNA Binding Studies

Other important examples of inorganic-based pharmaceuticals include metallocene platinum complexes, gold compounds and lithium antidepressants. In all these cases, research largely focused on elucidating the currently unknown mechanisms of action of these complexes. Attempts could be made on generating new and better inorganic drugs usually involved in synthesis of derivatives of the compounds known to be active with several coordination compounds, the extensive examples being the numerous platinum and other late transition metal analogues of Cis-DPP in the treatment of cancer. In principle, coordination compounds offer a great variety of shapes a reactivates for use in drug design. Exploring this potential however requires an understanding, at molecular level, of how the inorganic complexes can recognize, bind and perturb biologi macromolecules. These studies would enable us to understand the intercalations of the class coordination compounds. 195 Pt NMR spectroscopy would be used to study the binding of Cis I Trans-DPP to short duplex DNA fragments.

1HNMR Spectroscopic Investigation of DNA Binding

The tris (phenanthroline) metal complexes and their derivatives share several features that make them amenable to a systematic exploration of DNA site recognition. The complexes are coordinately saturated and inert to substitution. Hence, binding to DNA can be determined by a sum of noncovalent interactions rather than through metal-nucleotide coordination. The complexes are rigid in structure. Once some information regarding the orientation of the molecule with respect to the helix is available, information could be known with respect to the relative orientation of all the atoms of the molecule an the DNA helix. Various tris (phenanthroline) metal complexes would be prepared with a range (central metal ions). The metal center would therefore be varied to obtain a host of spectroscopic probe of the DNA complex interactions and phenanthroline

derivatives would be substituted while still preserving synthetic and spectroscopic parameters. The various systems being studied for the spectroscopic investigation are $[M(lig)^3]$ and $[M(lig)^2]$ (where M = series of transition metal ions like Au, Pt, Pd, Ru, Rh, etc. and Lig = Phenanthroline: substituted phenanthrolines like dap, damp etc. and mixed ligand complexes of the type $[M(L)2L1]^{2+}$ (where L = bipy, phenanthroline, and LI = Diamines viz ppz, dpp, DPPZ, phi, etc.) with a series of metal ions viz , Ru, Rh, etc. The results of these proposed studies would be of great significance because they illustrate how sensitive the emission spectrum is to the mode of DNA binding and because they illustrate the profound influence reaction kinetics can have in shaping the course of DNA binding, which in turn could give a clear view of how the coordination compounds offer a great variety of shapes and reactivates for use in the design of anticancer drugs. Metalloporphyrins with substituents on periphery Ru(Il) Rh(Il) complexes with polypyridines and I, 10-phenanthroline ligands also are of the same type of DNA-binding agents, where the structure, size and relative dispositions of the ligands on the coordinated sphere of the metal ions help direct the binding. These systems are widely used to probe DNA structure, and such studies that are proposed would help identify factors that shape the binding phenomenon.

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