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Metal Complexes of Curcumin for Cellular Imaging, Targeting, and Photoinduced Anticancer Activity

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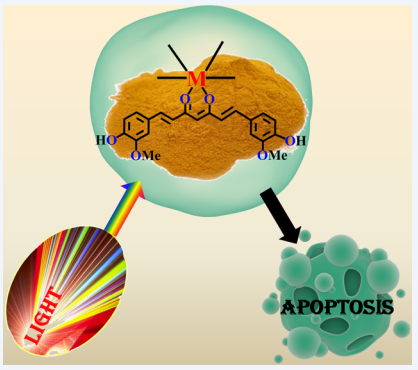
CONSPECTUS: Curcumin is a polyphenolic species. As an active ingredient of turmeric, it is well-known for its traditional medicinal properties. The therapeutic values include antioxidant, anti-inflammatory, antiseptic, and anticancer activity with the last being primarily due to inhibition of the transcription factor NF-κB besides affecting several biological pathways to arrest tumor growth and its progression. Curcumin with all these positive qualities has only remained a potential candidate for cancer treatment over the years without seeing any proper usage because of its hydrolytic instability involving the diketo moiety in a cellular medium and its poor bioavailability.



The situation has changed considerably in recent years with the observation that curcumin in monoanionic form could be stabilized on binding to a metal ion. The reports from our group and other groups have shown that curcumin in the metal-bound form retains its therapeutic potential. This has opened up new avenues to develop curcumin-based metal complexes as anticancer agents. Zinc(II) complexes of curcumin are shown to be stable in a cellular medium. They display moderate cytotoxicity against prostate cancer and neuroblastoma cell lines. A similar stabilization and cytotoxic effect is reported for (arene)ruthenium(II) complexes of curcumin against a variety of cell lines. The half-sandwich 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane (RAPTA)-type ruthenium(II) complexes of curcumin are shown to be promising cytotoxic agents with low micromolar concentrations for a series of cancer cell lines. In a different approach, cobalt(III) complexes of curcumin are used for its cellular delivery in hypoxic tumor cells using intracellular agents that reduce the metal and release curcumin as a cytotoxin.

Utilizing the photophysical and photochemical properties of the curcumin dye, we have designed and synthesized photoactive curcumin metal complexes that are used for cellular imaging by fluorescence microscopy and damaging the cancer cells on photoactivation in visible light while being minimally toxic in darkness. In this Account, we have made an attempt to review the current status of the chemistry of metal curcumin complexes and present results from our recent studies on curcumin complexes showing remarkable *in vitro* photocytotoxicity. The undesirable dark toxicity of the complexes can be reduced with suitable choice of the metal and the ancillary ligands in a ternary structure. The complexes can be directed to specific subcellular organelles. Selectivity by targeting cancer cells over normal cells can be achieved with suitable ligand design. We expect that this methodology is likely to provide an impetus toward developing curcumin-based photochemotherapeutics for anticancer treatment and cure.

INTRODUCTION

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione (diferuloylmethane), as an active ingredient of turmeric is well-known for its medicinal properties.[1,2] It is obtained from the rhizome of *Curcuma longa* L, the botanical name of turmeric. Curcumin is a lipophilic polyphenol. It is sparingly soluble in water. It shows stability in the acidic pH of the stomach.[3] Turmeric is used to treat many diseases particularly as an anti-inflammatory drug, for jaundice, colic pains, and wounds, etc.[3] It does not show any major side effects in low doses. A high dose of >100 mg (~3 g of turmeric) intake per day could cause diarrhea, skin rash, or nausea. Curcumin acts as an antiproliferative, antimetastatic, and antiangiogenic agent, inhibits carcinogenesis, and limits tumor growth.[3] Studies with *in vitro* models show that it induces apoptosis via mitochondrial pathways involving caspases and the Bcl-2 family of proteins.[3] Besides inhibiting angiogenesis, it interferes with the activity of NF-κB by enhancing the activity of the tumor suppressor protein p53. The anticancer properties are ascribed to its antioxidant and free-radical scavenging properties.[3] Curcumin inhibits the chemotherapeutic action of some drugs while enhancing the efficacy for other drugs. It is a potent anticancer agent but with a predicament. It has poor bioavailability and hydrolytic instability.[4] In summary, curcumin although acting against three important aspects of cancer, tumor promotion, angiogenesis, and tumor growth, has only limited utility due to its low bioavailability and hydrolytic instability.

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Curcumin (Hcur) can be stabilized on binding to a transition metal ion through its monoanionic enol form (Figure 1).[5−20]

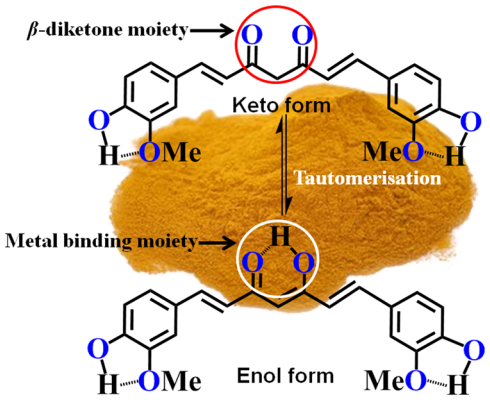

Figure 1. Keto−enol tautomeric forms of curcumin and the hydrogen bonding.

The curcumin concentration is very low in human plasma and other tissues (0.006 ± 0.005 μg mL^{-1}) even after an oral dose of 2 g kg^{-1} due to its hydrolytic instability.[3] Its hydrolytic stability increases on binding to a metal ion. While non-curcumin metal complexes are known for their anticancer properties, the utility of metal curcumin complexes as anticancer agents is yet to be fully realized.[21−24] Zinc(II) and half-sandwich ruthenium(II) complexes of curcumin are reported to show promising anticancer activity.[6−8] Complexes as selective delivery agents of curcumin in the cancer cells have also been reported recently.[9]

An important aspect of the curcumin dye is its spectral and photochemical properties.[25] It shows an absorption band within 410−430 nm and a fluorescence band within 460−560 nm in organic solvents. The spectral features remain similar on binding to the metal ion. This makes curcumin suitable as a ligand−photosensitizer in photoactivated chemotherapeutic studies. In addition, the complexes could be used for cellular imaging. The chemistry of photochemotherapeutic agents has gained importance with the successful use of Photofrin in photodynamic therapy (PDT) of cancer.[26] PDT is a non-invasive treatment modality of cancer in which the photo-exposed cancer cells are damaged without affecting the unexposed normal cells. Photofrin, with a hematoporphyrin core (Figure 2), on exposure to red light of 630 nm generates singlet oxygen ($^1O_2$) species.[26] The porphyrin-based dyes show skin photosensitivity and hepatotoxicity. Metal complexes showing photocytotoxicity in visible light with low toxicity in darkness are potentially suitable in PDT.[22−24,27,28] The curcumin complexes could be considered as a new class of metal-based photocytotoxic agents that are suitable for dual applications: (i) imaging the cells by fluorescence microscopy to study their cellular localization and (ii) damaging the photoexposed cells on irradiation with visible light. This Account presents the current status and future scope of metal-based curcumin complexes as potent cytotoxic and photo-cytotoxic agents.

CURCUMIN: ITS STABILITY AND PHOTOBIOLOGICAL ACTIVITY

Curcumin exists in keto−enol forms, and the equilibrium is dependent on the nature of the solvent.[25] Curcumin is sparingly soluble in water. It is soluble in organic solvents. The keto form dominates in acidic or neutral pH, while the enol form is favored in an alkaline pH where it shows only limited stability. Under physiological conditions (pH > 7.2), ∼90% of curcumin degrades within 30 min into several products, namely, *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane, and vanillin. It is also susceptible to degradation on exposure to light.[3,4] Curcumin can be stabilized in a solution of acidic pH or in the presence of antioxidants like glutathione, ascorbic acid, or NAC (*N*-acetyl-L-cysteine), and this is possibly related to the presence of two phenolic OH groups.[3] Curcumin thus suffers from its poor bioavailability besides being susceptible to degradation in light. Its degradation can be reduced in cell culture media having 10% fetal calf serum, in blood, on addition of phospholipid liposomes or BSA, on nanoencapsulation in organic polymers, or in liposomes or cyclodextrin.[3] A more facile and convenient way to enhance the solution stability without compromising its therapeutic efficacy is binding to a metal ion.

Curcumin is a photoactive compound with spectral features shown in Figure 3.[25] The emission quantum yield values range within 0.02−0.08.[25] The keto and enol forms have overlapping spectral features with similar absorption and emission spectra. The lifetime of the enol form is short due to intramolecular hydrogen bonding in nonpolar solvents and intermolecular hydrogen bonding in polar solvents providing a radiationless excited-state energy relaxation pathway.[25] The photophysical properties studied by Priyadarshini and co-workers indicate the enol form being ∼95% of the total amount with singlet state lifetime of <1 ns. The fluorescence and triplet quantum yields are very low due to strong hydrogen-bonding.[25] While the

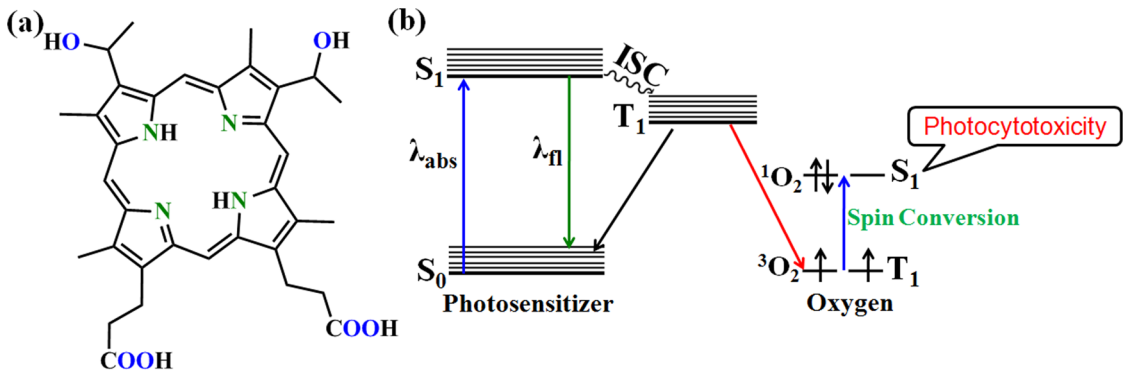

Figure 2. (a) Hematoporphyrin core. (b) Modified Jablonski diagram showing $^1O_2$ formation.

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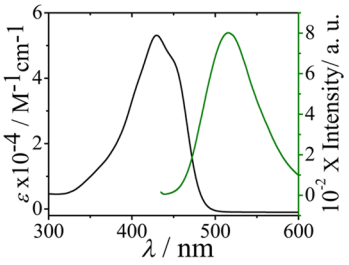

Figure 3. Absorption (black) and emission (green) spectra of curcumin in 10% aqueous DMF (λ_{ex} = 420 nm).

singlet excited state decay by a nonradiation process is facilitated by solvent and proton transfer, its triplet state absorbs at 720 nm and reacts with $^3O_2$ to generate $^1O_2$.[25] The photobiological activity of curcumin is thus related to its ability to generate ROS. The photoinduced ROS generation by curcumin leads to cancer cell death. As mentioned earlier, curcumin induces apoptosis via mitochondrial pathways involving caspases, the Bcl-2 family of proteins, and interferes with the activity of NF-κB.[3] Besides angiogenesis, it modifies several other factors involved in carcinogenesis. The low bioavailability, possibly due to its solution instability under physiological conditions, requires an effective method of delivery of curcumin in the cellular medium. This predicament could be one reason for the nonavailability of any literature reports on the PDT activity of curcumin.[26] The metal complexes are likely to provide a convenient way to overcome the challenges by delivering curcumin at the desired site.

METAL BINDING EFFECT ON CURCUMIN STABILITY AND CYTOTOXICITY

Binding to a metal ion enhances the stability of curcumin. Pucci, Valentini, and co-workers have shown that curcumin complexes of palladium(II) and zinc(II) are highly stable showing moderate anticancer activity.[5,6] Zinc(II) complexes of 2,2′-bipyridine derivatives have an absorption band within 408−450 nm and an emission band at 550 nm in DMSO (Figure 4). The photoluminescence quantum yield is ∼0.1,

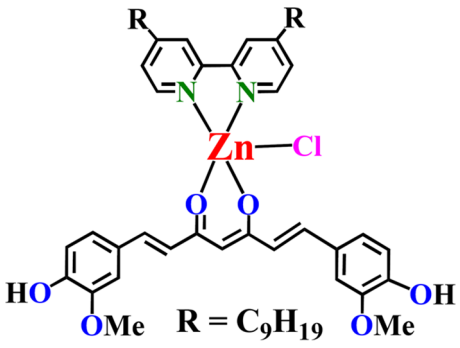

Figure 4. Perspective view of [(bpy-9)Zn(cur)Cl] based on the crystal structure.[6]

while it is only 0.03 for curcumin alone. Absorption spectral measurements with time have shown that the complexes are stable even after 48 h without any visual change in the shape and intensity of the band.[6] Curcumin alone undergoes considerable degradation in 48 h. While free curcumin induces 50% of cytotoxicity (IC_{50}) at concentrations <10 μM, the zinc(II) complexes are, however, less cytotoxic (IC_{50} = 12−37 μM) in prostrate cancer cells.[6] Based on the fluorescence properties of curcumin and its complexes, the alignment of the fluorescent groups is reported to be perpendicular to the helices with a possibility of intercalation. The anticancer activity of the curcumin complexes of palladium(II) and zinc(II) are similar. The ROS generated from curcumin play important roles in the apoptotic process with both pro- and antioxidant effects. Curcumin is also reported to act as a scavenger of ROS. It is thus of interest to see how the dual roles played by curcumin can be translated toward achieving cellular death by using the metal complexes.[5−8]

A mononuclear copper(II) curcumin complex is reported to show superoxide dismutase (SOD) activity.[29] The complex is redox active with a Cu(II)/Cu(I) couple at 0.4 V vs NHE. It scavenges superoxide radical at a rate of 1.97×10^5 M^{-1} s^{-1} in DMSO. This observation is of importance because the electron transport chain in mitochondria involves formation of superoxide radicals, which are also the precursor of hydroxyl radicals. Saladini and co-workers have reported a curcumin derivative to improve its properties by aromatic ring glycosylation.[30] They reported the activity in combination with cisplatin using glycosyl-curcuminoid drugs against both cisplatin-sensitive and cisplatin-resistant cell lines. Song and co-workers have shown that curcumin complexes of rare earth nitrates of Sm(III), Eu(III), and Dy(III) having 1,10-phenanthroline-5,6-dione show excellent antibacterial activity.[31] The proposed structure has three curcumin ligands and one phen-dione bound to lanthanide. Orvig and co-workers have shown that vanadyl, gallium, and indium complexes of curcumin are potent for medicinal applications.[32] The diacetylcurcumin (DAC) complexes $In(DAC)_3$ and $Ga(DAC)_3$ are less cytotoxic (IC_{50} = 20−30 μM) in mouse lymphoma cells compared with their curcumin analogues (IC_{50} = 5−10 μM). The vanadyl complex $[VO(cur)_2]$ is more effective as an anticancer agent than curcumin alone, while being nontoxic in vivo. Saladini and co-workers have reported iron(III) complexes of curcumin and diacetylcurcumin with significant stabilization of the curcumin ligand.[33]

Pelecanou and co-workers have used curcumin as the O,O-donor ligand and reported its complexes *fac*-$M(CO)_3$(cur)(imi) having imidazole and *fac*-$M(CO)_3$(cur)(isc).[34] The complexes were successfully used for selective staining of β-amyloid plaques of Alzheimer's disease (AD). The complexes labeled with fluorine-18 or technetium-99m as potential diagnostic tools for AD; gallium-68 (^{68}Ga)-labeled curcumin and curcuminoid complexes are studied as radiotracers for imaging cancer and AD. Asti and co-workers have reported $^{68}Ga(cur)_2^+$, $^{68}Ga(DAC)_2^+$, and $^{68}Ga(bDHC)_2^+$, where DAC and bDHC are diacetylcurcumin and bis(dehydroxy)curcumin, showing uptake in A549 lung cancer cells thus making these complexes useful as cancer-detecting radiotracers.[35] Ruthenium−arene complexes of curcumin are used for their anticancer activity. Caruso, Rossi, and co-workers have shown that [(p-cymene)Ru(cur)Cl] is effective against the colorectal tumor HCT116, breast MCF-7 and ovarian A2780 cells giving IC_{50} value of 13.98, 19.58, and 23.38 μM, respectively.[7] Interestingly, the complex is less toxic in the nonmalignant cell line MCF-10A (IC_{50} = 102.1 μM). Pettinari, Dyson, and co-workers have shown that organometallic (arene)ruthenium(II) RAPTA type complexes, where PTA is 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane, are highly effective against non-tumorous human embryonic kidney HEK293 cells, ovarian carcinoma A2780, and cisplatin-resistant A2780R cells giving

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IC_{50} values of ≤1.0 μM for the cancerous cells for an incubation time of 72 h (Figure 5).[8] The complexes are significantly less

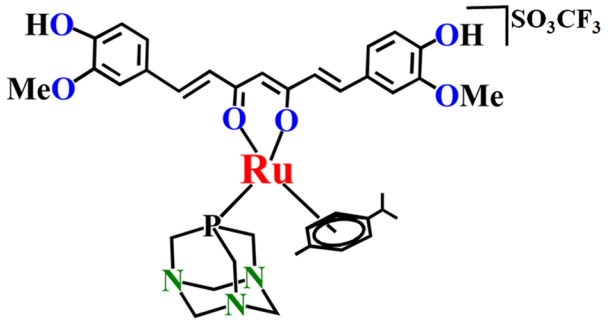

Figure 5. Perspective view of [Ru(cym)(cur)(PTA)](SO_3CF_3) based on the crystal structure.[8]

toxic for nontumorous cells. The complexes are more active than [Ru(cym)(cur)Cl].[7] The improved pharmacological properties of these curcumin-modified complexes are ascribed to the presence of the PTA ligand.

CELLULAR RELEASE OF CURCUMIN FROM COBALT COMPLEX

Transport and selective delivery of a chemotherapeutic drug to the tumor cells are important aspects of drug design and development. Hambley and co-workers have shown that cobalt curcumin complexes can be used to release curcumin with enhanced drug stability and tumor targeting and higher efficacy in hypoxic tumor cells.[9] The cobalt(III) complex, shown in Figure 6, is a prodrug that releases curcumin on reduction of

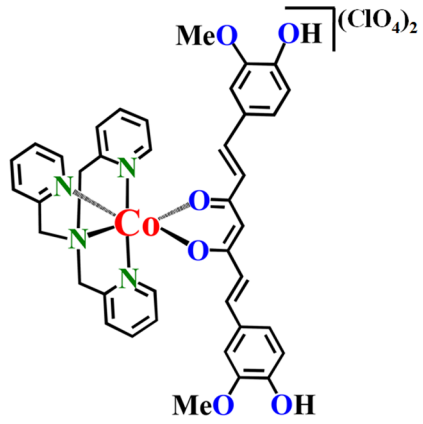

Figure 6. Proposed structure of [Co(tpa)(cur)](ClO_4)$_2$.[9]

the metal by cellular thiols to a cobalt(II) species. The Co(III)/Co(II) redox process being irreversible possibly involves a structural change. The complex shows modest cytotoxicity (IC_{50}: = 39 ± 4 μM in DLD-1 colon cancer cells), while curcumin alone is significantly more active (IC_{50} = 13 ± 2 μM). An interesting observation of this work is that the cell death is from the release of curcumin and not from the toxicity of the cobalt(III) complex. An analogous ruthenium(II) complex is reported to be more stable and release curcumin in reduced quantity with good cytotoxicity compared with the cobalt complexes.[9]

PHOTOCYTOTOXIC METAL COMPLEXES OF CURCUMIN

While the photobiological activity of curcumin is related to its solution stability and ability to generate singlet oxygen or radicals as the ROS, the low ϕ_f and ϕ_T values restrict its applications in PDT.[3,25] The metal complexes of curcumin show enhanced solution stability and are thus better suited for phototherapeutic applications.[5−20] The zinc(II) and palladium(II) complexes and the ruthenium(II) organometallic complexes are studied for their cytotoxic activities but without any photoirradiation.[5−8] A general observation is that these complexes are less cytotoxic than curcumin itself.[5−8] The positive aspects of these complexes are that they show remarkable stability of curcumin on binding to the metal and thus can possibly increase the bioavailability of curcumin. Our interest in this chemistry is to explore the photocytotoxic potential of the complexes in visible light. Our objective toward designing the ternary curcumin complexes where the metal is bound to an ancillary ligand is to achieve (i) selectivity, that is, targeting the cancer cells over normal cells, (ii) higher cellular uptake in the cancer cells over normal cells, (iii) targeting different cellular organelles to enhance the efficacy of the complexes, (iv) cellular imaging, and (v) generation of different types of ROS (Figure 7).

Figure 7. Ternary structure [{R(X∩Y)}M(cur)]$^{n+}$, where M is a transition metal and R is the cell targeting, ROS generating, and fluorophore moiety.

An oxovanadium(IV) complex [VO(cur)(dppz)Cl] having dipyridophenazine (dppz) as the ancillary ligand was found to be very stable in 1:1 DMSO−Tris buffer (pH = 7.2, 37 °C) even up to 48 h, while curcumin alone underwent almost complete degradation in 4 h incubation time (Figure 8).[10]

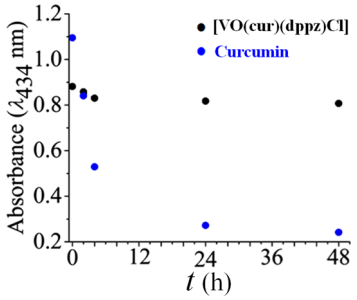

Figure 8. Plots showing the time dependent absorbance of curcumin (Hcur, blue) and [VO(cur)(dppz)Cl] (black) in 1:1 DMSO−Tris buffer (pH = 7.2, 37 °C). Reproduction with permission from ref 10. Copyright 2012 Royal Society of Chemistry.

Hence, binding to the VO^{2+} moiety arrests the hydrolytic cleavage of curcumin and enhances its hydrolytic stability. The crystal structure of the 1,10-phenanthroline (phen) analogue has a V═O bond (~1.54 Å) trans to the significantly long V−Cl bond (~2.78 Å) making the complexes 1:1 electrolytic in aqueous DMF with dissociation of the chloride ligand forming a stable five-coordinate structure as evidenced from the ESI-MS

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data (Figure 9). The dppz complex is remarkably photocytotoxic in HeLa cells (IC_{50} = 3.3 μM) in visible light (400−700 nm), while being less toxic in darkness (IC_{50} > 50 μM). The curcumin dye alone has IC_{50} values of 8.2 μM in light and 85 μM in the dark under similar experimental conditions. The dppz ligand is less toxic (IC_{50} = 60 μM in light and >100 μM in the dark). The fluorescence property of curcumin has enabled cellular localization study of the complex. It has cytoplasmic localization with marginal nuclear uptake.

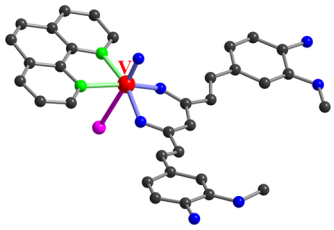

Figure 9. Crystal structure of [VO(cur)(phen)Cl]. Reproduction with permission from ref 10. Copyright 2012 Royal Society of Chemistry.

The diglucosylcurcumin (scur) analogue [VO(scur)(dppz)-Cl] shows higher cellular uptake.[11] The sugar-appended complex is highly photocytotoxic in visible light (IC_{50} < 5 μM) in HeLa, MCF-7, and HaCaT cells with low dark toxicity. The complexes, as avid binders to DNA, exhibit plasmid pUC19 DNA cleavage activity in red light with formation of hydroxyl radicals. The cellular death follows an apoptotic pathway involving ROS. A related oxovanadium(IV) complex [VO(pyphen)(cur)](ClO_4) in which the chloride and dppz ligands are replaced by a tridentate 2-(2′-pyridyl)-1,10-phenanthroline (pyphen) base shows significant photocytotoxicity in HeLa and MCF-7 cancer cells in visible light (400−700 nm) with IC_{50} values of ∼5 μM, while being less toxic in normal fibroblast 3T3 cells in light (IC_{50} ≈ 21 μM) and in darkness (IC_{50} > 50 μM).[12] This complex displays both nuclear and cytosolic localization.

Curcumin (cur) and glycosylated curcumin (scur) complexes of lanthanide (Ln) ions, namely, La(III) and Gd(III), having terpyridyl (R-tpy) ligands are remarkably photocytotoxic in HeLa cells.[13] Complexes [Ln(R-tpy)(cur/scur)(NO_3)$_2$] are stable without showing any degradation of the curcumin ligand (Figure 10). The crystal structures of the complexes show the presence of tridentate R-tpy, bidentate anionic cur, and two bidentate nitrate ions. They are 1:1 electrolytic in aqueous DMF with dissociation of one nitrate ion as evidenced from the ESI-MS and conductivity data. The IC_{50} values of the pyrenylterpyridine complexes are <5 μM in light of 400−700 nm, while remaining essentially nontoxic in the dark (IC_{50} >100 μM). The glycosylated complexes are less effective (IC_{50} ≈ 20 μM). The complexes show significant cellular uptake mainly in the nucleus. No change in the nuclear morphology takes place, indicating that they are not harmful in darkness. The results are significant because such complexes can be used for cellular imaging and also for cell damage on light irradiation, while the lanthanide-based MRI agents are suitable for only tumor detection and not for any tumor damage. Interestingly, the ROS formed on photoactivation of these complexes are singlet oxygen ($^1O_2$) and hydroxyl ($OH^•$) radicals. Observation of different types of ROS may be due to the presence of a pyrenyl group and cur/scur as two different photoactive moieties.

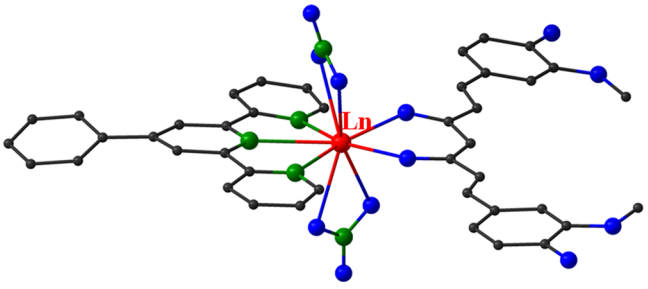

Figure 10. Crystal structure of [Ln(ph-tpy)(cur)(NO_3)$_2$] [Ln(III) = La(III), Gd(III)].[13]

Ternary copper(II) and oxovanadium(IV) complexes of curcumin or glycosylated curcumin, shown in Figure 11, having *N*-ferrocenylmethyl-L-amino acid (Fc-aa), dipicolylamine, or terpyridyl ligands are significantly photocytotoxic in different cancer cells.[14−16] The Fc-aa copper(II) complexes of curcumin are photocytotoxic in HeLa and MCF-7 cells giving respective IC_{50} value of ∼4 and ∼14 μM, while being less toxic in darkness (IC_{50} > 45 μM in HeLa; >74 μM in MCF-7).[14] The proposed structures of the Fc-TrpH and Fc-TyrH complexes are square-planar. The Fc-MetH complex is square-pyramidal. The rigid geometry of the complexes seems to be responsible for making the copper(II) center redox-inactive, while the ferrocenyl moiety is redox active (Fc^+−Fc couple ∼0.45 V vs SCE in MeCN−0.1 M TBAP). The cellular death is via an apoptotic pathway. The complexes localize predominantly in the cytosol.[14] The change of ferrocenyl conjugates from L-amino acids to the terpyridyl (tpy) group changed the photophysical properties of the curcumin complexes. The Fc-tpy complexes have an intense metal-to-ligand charge transfer (MLCT) band near 600 nm in [VO(Fc-tpy)(cur/bDHC/bDMC)](ClO_4), where bDHC and bDMC are bis-dehydroxycurcumin and bis-demethoxycurcumin.[16] The redox active curcumin complex with Fc^+/Fc at ∼0.65 V (vs SCE in DMF−0.1 M TBAP) is significantly photocytotoxic in visible light (400−700 nm) in HeLa (IC_{50} ≈ 2.4 μM) and Hep G2 (IC_{50} ≈ 11 μM) cells with low dark toxicity (IC_{50} > 50 μM).[16] The curcumin derivatives are less active. The curcumin complex is also less active in normal 3T3 cells (IC_{50} ≈ 22 μM in visible light). The complexes localize in both the cytosol and nucleus. The DNA photocleavage data suggest formation of hydroxyl radicals as the ROS. The ferrocenyl moiety plays an important role as is evidenced from ∼7-fold higher cellular uptake of the Fc-tpy complex than its phenyl analogue.[16] This may be due to higher lipophilicity of the ferrocenyl moiety. The ferrocenyl conjugate of dipicolylamine (Fc-pic) has lower photocytotoxicity than its Fc-tpy analogue.[15] This is possibly due to the absence of the MLCT band in the Fc-pic complex near 600 nm. The Fc-pic complexes localize in both cytosol and the nucleus. From the above discussion, it is apparent that while curcumin as such is not suitable under PDT conditions due to its hydrolytic instability, its metal complexes show significantly enhanced (of ∼5−10 times) photocytotoxicity in visible light along with greater solution stability.

MITOCHONDRIAL LOCALIZATION

Chemotherapeutic drugs like cisplatin and its analogues target the nuclear DNA. In contrast, the PDT drug Photofrin shows mitochondrial localization. Drugs targeting mitochondria and effecting cell death via apoptotic pathways are likely to provide an alternate viable strategy in anticancer drug development.[26] Curcumin displays its anticancer activity via the intrinsic

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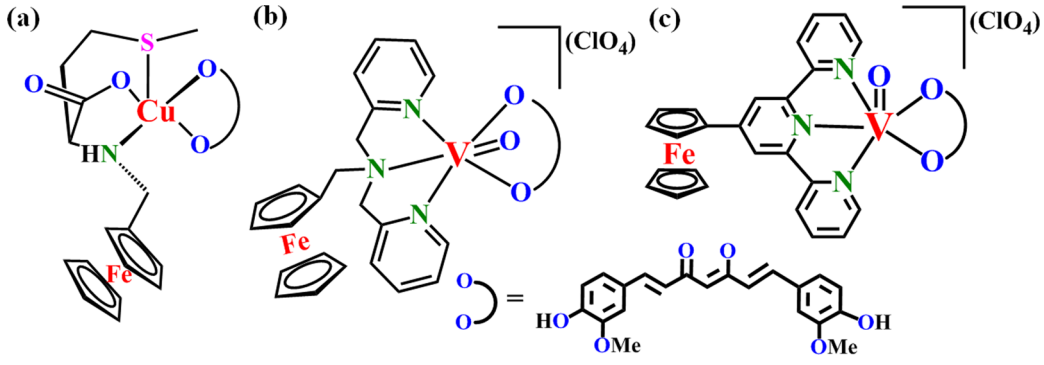

Figure 11. Proposed structures of [Cu(Fc-met)(cur)] (a), [VO(Fc-pic)(cur)](ClO_4) (b), and [VO(Fc-tpy)(cur)](ClO_4) (c) with a pendant ferrocenyl (Fc) moiety.[14−16]

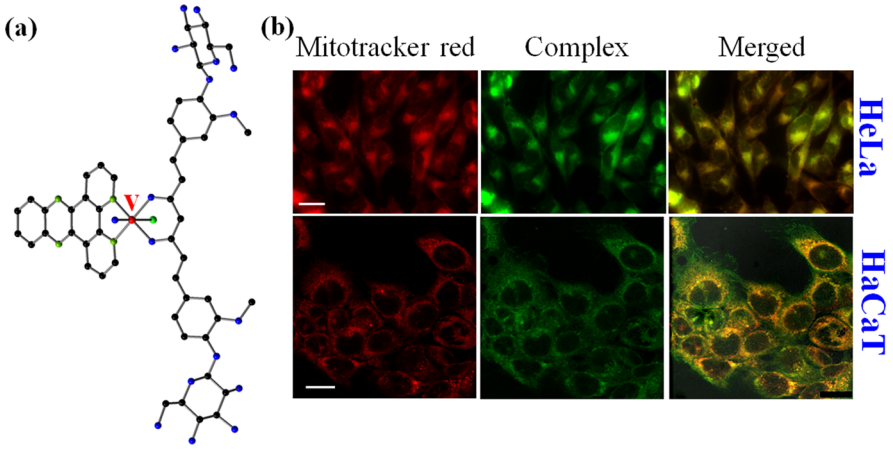

Figure 12. Energy optimized structure of [VO(scur)(dppz)Cl] (a) and its (10 μM) confocal images (b) using MitoTracker deep red (MTR, 0.05 μM) in HeLa and HaCaT cells after 4 h incubation (Hscur = diglucosylcurcumin).[11] Scale bar: 20 μm. Reproduction with permission from ref 11. Copyright 2015 Royal Society of Chemistry.

pathway.[3] Curcumin being a fluorescent molecule, its complexes could be used to study their cellular localization.[10−20] The curcumin metal complexes show both nuclear and cytosolic localization in varying degrees. Curcumin alone shows cytosolic localization, and its complexes are thus of interest for targeting subcellular organelles other than the nucleus. Ternary complexes [VO(cur/scur)(dppz)Cl], shown in Figure 12a), localize primarily in the cytosol.[11] Further studies have revealed mitochondrial localization as evidenced from the merged fluorescence microscopic images of the complexes (green emitter) and the MitoTracker deep red (MTR) (Figure 12b). The complex shown in Figure 13a having an NNN-donor ancillary ligand with a naphthalimide (napth) pendant is photocytotoxic ($IC_{50} \approx 5.5$ μM) in HaCaT and MCF-7 cells in visible light of 400−700 nm, while being less toxic in the dark ($IC_{50} > 50$ μM).[17] Dual staining with red emitting MTR shows its mitochondrial localization giving yellow as the merged color. Interestingly, the ancillary ligand alone localizes in the nucleus. An analogous complex having phenylterpyridyl ligand with a pendant triphenylphosphonium moiety (TPP-phtpy) also shows mitochondrial localization in MCF-7 cells, while no specific cellular localization is reported for the control complex lacking the TPP moiety.[18]

The ternary oxovanadium(IV) complex [VO(cur)(acdppz)-Cl], shown in Figure 13b, having (acridinyl)dipyridophenazine (acdppz) ligand, is remarkably photocytotoxic in visible light (400−700 nm) giving IC_{50} values of 0.2 μM in HaCaT and 0.7 μM in HeLa cells, while being less toxic in darkness ($IC_{50} > 10$ μM).[19] It also shows mitochondrial localization. The diglucosylcurcumin analogue is, however, cytotoxic in both dark and light giving IC_{50} of 4.2 and 0.3 μM, respectively. It localizes predominantly within the nucleus. The cell death is via an apoptotic pathway by light-generated cellular ROS. The complexes show excellent plasmid DNA photocleavage activity in red light (705 and 785 nm) forming singlet oxygen as the

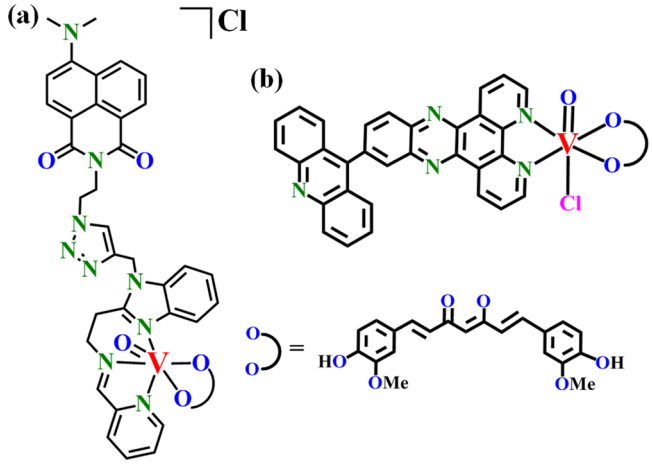

Figure 13. Proposed structure of [VO(napth-py-aebmz)(cur)]Cl (a)[17] and perspective view of [VO(cur)(acdppz)Cl] (b) based on modeling.[19]

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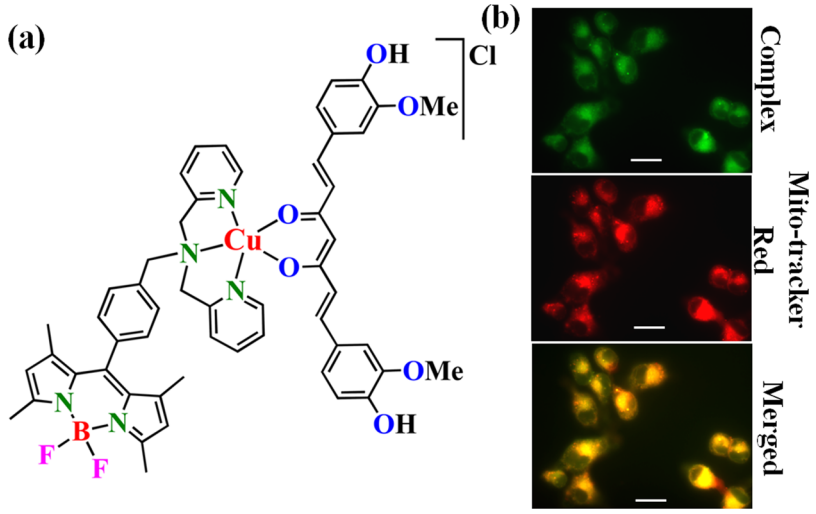

Figure 14. (a) The proposed structure of [Cu(L)(cur)]Cl of a dipicolylamine ligand (L) having a BODIPY moiety. (b) Fluorescence microscopic images of the HeLa cells with this complex (10 μM) and MitoTracker Red (MTR, 0.05 μM) on 4 h incubation.[20] Scale bar = 20 μm. Reproduction with permission from ref 20. Copyright 2015 Royal Society of Chemistry.

ROS, while the ligands are inactive. This curcumin complex is remarkable in fulfilling several basic requirements of PDT: (i) singlet oxygen as the ROS, (ii) mitochondrial localization, (iii) apoptotic cell death, (iv) photocytotoxicity in visible light with relatively low dark toxicity (good PDT effect), and (v) near-IR light-induced DNA cleavage activity. Moreover, this complex with its prolonged solution stability and inactivity in darkness can overcome the side effects that are known for Photofrin (namely, skin photosensitivity and hepatotoxicity) arising due to oxidative degradation of the tetrapyrrolic core.[26]

Complexes [Cu(L)(cur)]Cl, shown in Figure 14a, where L is dipicolylamine with a pendant BODIPY moiety and its diiodo derivative, are photocytotoxic in visible light in HeLa cells.[20] The IC_{50} value of the diiodo species is 3.8 μM in light (400−800 nm), while being less toxic in the dark (IC_{50} = 32 μM). The noniodo species being emissive is used for cellular imaging study. Dual staining using nuclear staining dye Hoechst 33342 shows its cytosolic localization. A similar experiment using MTR exhibits significant mitochondrial localization as shown in Figure 14b. These copper(II) curcumin complexes having BODIPY pendant show both PDT effect and mitochondrial localization. The light-induced cellular death is via an apoptotic pathway generating ROS.

■ CONCLUSIONS AND FUTURE SCOPE

The anticancer activity of curcumin is limited due to its low bioavailability and susceptibility for hydrolytic degradation under physiological conditions. These predicaments limit its application, although it is well-known for its wide range of medicinal properties. Curcumin being fluorescent could be used for cellular imaging, although the fluorescence intensity diminishes with time due to its degradation. The therapeutic efficacy of curcumin can be realized in its metal-bound form as can be seen from the data listed in Table 1. The IC_{50} values show that binding of curcumin to a metal increases its photocytotoxicity. The enhancement is ∼5 times for the oxovanadium(IV) and copper(II) complexes. In addition, binding to metal increases its solution stability in a significant manner. The literature reports show that zinc and palladium complexes increase the stability of the dye, but the anticancer activity of the complexes remains at the modest level. The

Table 1. IC_{50} (μM) Values of Curcumin, Its Complexes, and Photofrin

	IC_{50} value			
compound	light	dark	cell line[a]	ref
curcumin (Hcur)	8.2 ± 0.2[b]	85 ± 4[c]	HeLa	10
[Zn(phen)(cur)Cl]		8.75[d]	SH-SY5Y	6
[(p-cymene)Ru(cur)Cl]		23.4 ± 3.3[e]	A2780	7
[Ru(cym)(bdcurc)(PTA)]PF_6		0.14 ± 0.05[e]	A2780	8
[Co(tpa)(cur)]$(ClO_4)_2$		39 ± 4[e]	DLD-1	9
[VO(scur)(dppz)Cl]	1.5 ± 0.2[b]	45 ± 1[c]	HeLa	11
[VO(pyphen)(cur)](ClO_4)	3.4 ± 0.3[b]	>50[c]	HeLa	12
[La(pytpy)(cur)$(NO_3)_2$]	4.6 ± 0.6[b]	>100[c]	HeLa	13
[Cu(Fc-met)(cur)]	2.9 ± 0.3[b]	47.8 ± 2.1[c]	HeLa	14
[VO(cur)(acdppz)Cl]	0.7 ± 0.1[b]	>10[c]	HeLa	19
Photofrin[f]	4.3 ± 0.2	>41	HeLa	26

[a]HeLa, human cervical cell; SH-SY5Y, human neuroblastoma cell; A2780, human ovarian cell; DLD-1, colorectal cell. [b]For 4 h incubation in darkness followed by 1 h light exposure (400−700 nm, 10 J cm^2). [c]For 4 h incubation in darkness. [d]For 24 h incubation in darkness. [e]For 72 h incubation in darkness. [f]Red light of 633 nm. Incubation time of 24 h.

organometallic half-sandwich ruthenium(II) complexes of curcumin show promising anticancer activity. Hambley and co-workers have shown a novel way to deliver curcumin in hypoxic tumor cells by using cobalt(III) complexes that undergo reduction by cellular thiols releasing curcumin. This can enhance the therapeutic potential of curcumin. Metal complexes of curcumin are suitable for dual effects, that is, cellular imaging utilizing the fluorescence properties of curcumin and damaging the cancer cells on their photoactivation in visible light. Our recent efforts toward developing the chemistry of photocytotoxic curcumin complexes are highlighted in this Account. The ternary structures are designed with a variety of ancillary ligands. The complexes are used for targeting different subcellular organelles, to selectively target cancer cells over the normal cells, and for enhancing the cellular

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uptake besides generation of different ROS, namely, singlet oxygen and hydroxyl radicals. We have shown that what could not be achieved by using curcumin alone or its metal complexes in darkness could be achieved by using visible light as an activator. In summary, we have highlighted three major aspects of this emerging chemistry: (i) hydrolytic stability of curcumin on binding to a metal ion, (ii) observation of remarkable PDT effect of the complexes in visible light, and (iii) utility of the complexes in cellular imaging where the metal-bound curcumin acts as a fluorophore with its green emission property (Figure 15). The future scope of this chemistry is based on the

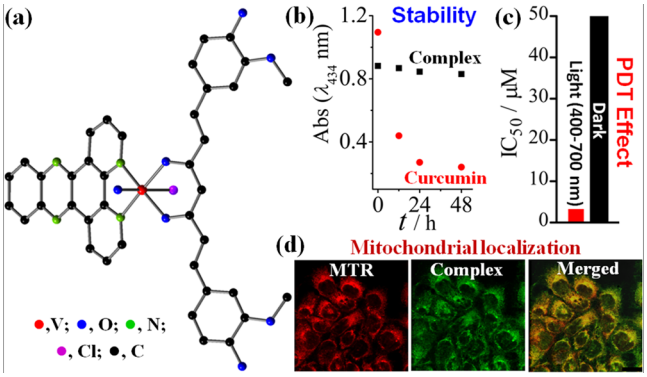

Figure 15. Major aspects of a metal−curcumin complex (a): stabilization of the dye on binding to a metal ion (b), remarkable PDT effect (c), and cellular imaging (d).

methodology developed by us to provide new avenues for designing biocompatible curcumin metal complexes as potential photochemotherapeutic agents.

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Notes

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ABBREVIATIONS

acdppz, 11-(9-acridinyl)dipyrido[3,2-*a*:2′,3′-*c*]phenazine; Bcl-2, B-cell lymphoma 2; bdcurc, bisdemethoxycurcumin monoanion; bDHC, bis(dehydroxy)curcumin; bDMC, bis-demethoxycurcumin; BODIPY, borondipyrromethene; bpy-9, 4,4′-dinonyl-2,2′-bipyridine; cur, curcumin monoanion; cym, cymene; DAC, diacetylcurcumin; DCFDA, 2′,7′-dichlorofluorescin diacetate; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine; ESI-MS, electrospray ionization-mass spectrometry; Fc, ferrocenyl; Fc-MetH, ferrocenylmethyl-L-methionine; Fc-pic, ferrocenylmethyl-bis(2-pyridylmethylamine); Fc-tpy, 4′-ferrocenyl-2,2′:6′,2″-terpyridine; Fc-TrpH, ferrocenylmethyl-L-tryptophan; Fc-TyrH, ferrocenylmethyl-L-tyrosine; imi, imidazole; isc, isocyanocyclohexane; IC_{50}, half maximal inhibitory concentration; L, dipicolylamine ligand with BODIPY moiety; MTR, MitoTracker deep red; NAC, *N*-acetyl-L-cysteine; napthpy-aebmz, naphthalimide conjugated 2-(1*H*-benzimidazol-2-yl)-*N*-(pyridin-2-ylmethylene)ethanamine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NHE, normal hydrogen electrode; PDT, photodynamic therapy; phen, 1,10-phenanthroline; Ph-tpy, 4′-phenyl-2,2′:6′,2″-terpyridine; pyphen, 2-(2′-pyridyl)-1,10-phenanthroline; pytpy, 4′-(1-pyrenyl)-2,2′:6′,2″-terpyridine; RAPTA, 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane; ROS, reactive oxygen species; SCE, saturated calomel electrode; scur, diglucosylcurcumin monoanion; SOD, superoxide dismutase; TBAP, tetrabutylammonium perchlorate; tpa, tris(2-methylpyridine)amine; TPP, triphenylphosphonium ion

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