Evaluation of Triazole-Chelated Lanthanides as Chemically Stabile Bioimaging Agents

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ABSTRACT: Europium (Eu), dysprosium (Dy), samarium (Sm), and terbium (Tb) complexes were prepared using the neutral tridentate chelator 2,6-bis(1-benzyl-1,2,3-triazol-4-yl)pyridine and one equivalent of each lanthanide salt. The physicochemical, aerodynamic, and *in vitro* cellular properties of each lanthanide metal complex were studied to determine their viability as cell surface fluorescent probes. Each compound was characterized by electrospray ionization mass spectroscopy (ESI–MS), ultraperformance liquid chromatography (UPLC), differential scanning calorimetry (DSC), and thermogravimetic analysis (TGA). Upon excitation at 320 nm each complex displayed characteristic lanthanide-based fluorescence emission in the visible wavelength range with stokes shifts greater than 200 nm. Each complex was found to be chemically stable when exposed to pH range of 1–11 for 72 h and resistant to photobleaching. To simulate pulmonary administration of these fluorophores, the aerodynamic properties of the Eu and Tb complexes were determined using a next generation impactor (NGI). This measurement confirmed that the complexes retain their fluorescence emission properties after nebulization. Cellular cytotoxicity was determined on A-549 lung cancer cell line using methylthiazol tetrazolium (MTT) cytotoxicity assay at 24, 48, and 72 h postexposure to the complexes. The complexes showed a dose and time-dependent effect on the percent viability of the cells. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2589–2598, 2013

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INTRODUCTION

Luminescent compounds have helped "shed light" on the structure and function of biological systems, as fluorescence spectroscopy has impacted diverse fields including immunology, biochemistry, biophysics, biotechnology, and medicine.[1,2] The many fluorescent probes currently available commercially include organic dyes (e.g., fluorescein, rhodamine, etc.), quantum dots, biological fluorophores (e.g., green fluorescent protein), transition metal ion complexes, and lanthanide metal ion complexes,[3–7] each with inherent advantages and disadvantages when employed as bioimaging agents.

The lanthanides are the f-block elements extending from lanthanum (Ln, atomic number 57) through lutetium (Lu, atomic number 71). Their electronic configurations have a xenon core with varying numbers of 4f valence electrons [(Xe)4fn]. The shielding of the atomic core by 4f valence electrons accounts for the characteristic chemical, magnetic, and spectral properties of this series.[8] Many lanthanide metals display intense and sharp fluorescence emission bands but are themselves photophysically inert and cannot be directly excited. Therefore, luminescent lanthanide compounds require ligands able to serve as antenna by absorbing excitation energy and then transferring this energy to the lanthanide ion,[2,9] leading to lanthanide-based fluorescence emission.[10–15] Among the many examples of organic ligands able to serve this role are triazole-based chelators.[16–18]

Because of their unique fluorescence properties, lanthanide complexes have been useful in immunoanalysis, tissue-specific imaging, and detection of single molecules in living cells.[12–14,19–21] Other applications include molecular diagnostics, drug discovery,

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and tumor therapy.[14,22–26] For example, the antitumor activity of quercetin was enhanced when chelated to lanthanide metal ion because of direct interaction with DNA,[20,27] fluorescent nanoparticles of lanthanide metal complexes were used for optical imaging in cancer therapy,[28] and lanthanide metal ion chelates were used for time-resolved immunoassays and dissociation enhanced fluoro-immunoassays.[29]

Advantages inherent to lanthanide complexes relative to other classes of fluorescence probes include their sharp and intense emission bands, long fluorescence lifetimes (submicrosecond to millisecond range), large Stokes shifts (>150 nm), resistance to photobleaching, and high quantum yields.[2,10,11,29–33] The broad utility of lanthanide metal complexes as imaging agents is enabled by spectral output ranging from ultraviolet [e.g., gadolinium (Gd)], to visible light [e.g., samarium (Sm), europium (Eu), terbium (Tb), dysprosium (Dy), and thulium (Tm)], to near-infrared [e.g., neodymium (Nd), erbium (Er), and ytterbium (Yb)]. The magnetic properties of some metals (e.g., Gd), additionally allow their use as magnetic imaging probes. Unlike the transition metal series, lanthanide metals largely share the same patterns of reactivity regardless of lanthanide identity.[34] Such interchangeable reactivity, combined with the wide-ranging physical properties imparted by lanthanide identity, makes lanthanide-based probes attractive systems by which to develop new imaging agents with variable readouts.

There are some important features for devising a probe for use in living cells. The probes should maintain their integrity and function inside the cells. They should be resistant to enzymatic degradation, effectively function in relevant biological media[9] and should minimally photobleach and quench.[35–37] Furthermore, the probes should not disturb or alter the homeostasis of the cells and should not affect the proliferation or viability of the cells.[35] This is particularly important for probes intended for pulmonary applications. The clearance of inhaled particles from lung tissue can occur by mucociliary clearance and ingestion, systemic absorption, mechanical translocation, pulmonary biometabolism, phagocytosis by alveolar macrophages, or biodegradation in lung spaces. Probes could then be designed and engineered as inhaled particles to either avoid or conversely target specific pulmonary clearance mechanisms for various imaging utilities. Incubation of probes with cells has been reported in the literature to alter the cellular permeability.[38–41] Therefore, probes should be designed in a way that they should not change the permeability of the cellular membrane.[9,35] The probe should also be highly selective for its target substrate[9] and should cross the membrane and localize to a particular organelle.[35]

This report describes the preparation and characterization of four lanthanide metal complexes, three of which are unreported in the chemical literature. These were evaluated for their potential to serve as fluorescent probes in biological environments, their amenability toward pulmonary administration, and their general cytotoxicity. In this study, hydrophobic benzylic substituents were used as a simple model system to define the stability of such organometallic constructs under biochemically relevant conditions. Success in defining such complexes as stable fluorescent probes would promote this class of compounds as attractive new tools for bioimaging wherein probe emission and target binding affinity could each be modularly varied by lanthanide selection and peripheral ligand functionalization, respectively.

MATERIALS

Acetonitrile, denatured ethyl alcohol, tetrahydrofuran (THF), hexanes, isopropanol, *tert*-butanol, ethyl acetate, acetone, 14.8 N ammonium hydroxide, sodium dodecyl sulfate (SDS), Dulbelcco's phosphate buffered saline, and sodium chloride were used as purchased from Fisher Scientific (Pittsburgh, Pennsylvania). Copper sulfate was used as purchased from Strem Chemicals, Inc. (Newburyport, Massachusetts). 2,6-Bis(trimethylsilylethynyl)pyridine was prepared as previously reported.[42] Benzyl bromide, sodium azide, and sodium ascorbate, dimethyl sulfoxide, cyclohexanone, ethanol absolute 200 proof, 12 N hydrochloric acid, sodium bicarbonate, thiazolyl blue tetrazolium bromide, dimethyl formamide (DMF), and octanol were used as purchased from Sigma–Aldrich (St. Louis, Missouri). Pyrolidine, magnesium chloride, and diethyl ether were purchased from Acros Organic (New Jersy). Ham's F-12 K medium and trypsin—ethylenediaminetetraacetic acid (EDTA) were purchased from Cellgro Mediatech (Manassas, Virginia); fetal bovine serum (FBS), penicillin/streptomycin 10/10 100×, L–glutamine 100× from Atlanta Biologicals Lawrenceville, Georgia); sodium pyruvate and nonessential amino acids mixture from Lonza Biowhittaker (Suwanee, Georgia). A549 lung cancer cell line was purchased from American Type Culture Collection (Manassas, Virginia).

METHODS

Ligand Synthetic Route

Preparation of the 2,6-bis(1-benzyl-1,2,3-triazol-4-yl)pyridine chelator (L) has been previously reported in the literature.[42,43] Preparation of EuL_3OTf_3 complexes by reaction of $Eu(OTf)_3$ salts with three

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