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Synthesis and characterization of sulfonamide derivatized di-(2-picolyl)amine ligands and their rhenium tricarbonyl complexes towards fluorescent imaging

T Darshani[1], I C Perera[2], N T Perera[1]*

[1]*Department of Chemistry, University of Sri Jayewardenepura, Sri Lanka*

[2]*Department of Zoology and Environment Science, University of Colombo, Sri Lanka*

**Email: theshi@sjp.ac.lk*

The synthesis and characterization of four novel compounds are reported in this study; $N(SO_2)$(1-nap)dpa (L1), $N(SO_2)$(2-nap)dpa (L2), *fac*-[$Re(CO)_3$($N(SO_2)$(1-nap)dpa)]PF_6 (C1), *fac*-[$Re(CO)_3$($N(SO_2)$(2-nap)dpa)]PF_6 (C2) to evaluate their application as imaging agents. The photophysical properties and bio applicability of the novel compounds as well as of two previously synthesized compounds; $N(SO_2Me_2Nnap)$dpa (L3) and *fac*-[$Re(CO)_3$($N(SO_2Me_2Nnap)$dpa)]PF_6 (C3) are reported herein. All the compounds (Figure 1) were characterized by X-ray diffraction studies, ^{1}H NMR, FT-IR, UV-Vis and fluorescence spectroscopies.

X-ray crystallographic analyses of the ligands confirmed the formation of the expected sulfonamide appended di-(2-picolyl)amine based ligands. The two naphthalene derivatized ligands, L1 and L2, were crystalized in monoclinic form while the triclinic form was obtained for L3. Formation of C1, C2 and C3 complexes were confirmed by ^{1}H NMR studies in DMSO-d_6 in which the singlet peak in the spectra of the free ligands designated for the methylene protons (~4.7 ppm) appeared as two doublets (*endo*- and *exo*-CH) upon binding to the metal (Table 1). In the FTIR spectra of the ligands, peaks due to S-N stretching vibrations obtained at 918-995 cm^{-1} have shifted to lower wavenumbers in the spectra of the metal complexes. The peaks in the absorption spectra of the metal complexes have shifted significantly compared to the respective free ligands. The absorption spectra of C1 and C3 show a bathochromic shift while C2 shows a hypsochromic shift compared to the respective free ligands. With the exception of C2, all other compounds displayed promising photophysical properties with intense fluorescence peaks (L1: 338 nm, L2: 343 nm, L3: 525 nm, C1: 335 nm and C3: 535 nm). Among them, fluorescence spectra of L2, L3 and C3 showed remarkably high intensities even at low compound concentrations of 0.01 mM. Fluorescence microscopy images generated on human lymphocytes incubated with the synthesized compounds showed excellent cellular uptake for L3 and C3. Hence, fluorescence was observed at low concentrations of the compounds. Cell viability was not affected at these concentrations. Furthermore, localization of C3 in cytoplasmic membrane and cell nucleus of lymphocytes could be observed. In conclusion, there is a great potential of utilizing L3 and C3 in bio imaging applications.

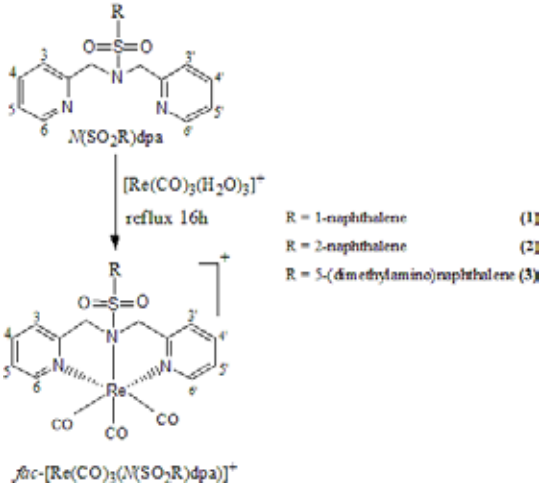

Figure 1. Synthetic route of metal complexes

Table 1. Comparison of ^{1}H NMR shifts of the synthesized compounds

	H6/6'	H5/5'	H4/4'	H3/3'	CH_2
$N(SO_2)$(1-nap)dpa	8.32	7.14	7.55	7.10	4.70
[$Re(CO)_3$($N(SO_2)$(1-nap)dpa)]PF_6	8.90	7.47	7.98	7.37	5.66, 4.52
Δδ (ppm) of C1	(+) 0.58	(+) 0.33	(+) 0.43	(+) 0.27	
$N(SO_2)$(2-nap)dpa	8.32	7.15	7.63	7.29	4.60
[$Re(CO)_3$($N(SO_2)$(2-nap)dpa)]PF_6	8.89	7.47	8.00	7.46	5.67, 4.59
Δδ (ppm) of C2	(+) 0.57	(+) 0.32	(+) 0.37	(+) 0.17	

Keywords
imaging agents, sulfonamide complexes, Rhenium tricarbonyl, di-(2-picolyl)amine ligands

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A novel immunoanalytical method for obesity biomarker detection using antibody functionalized silver nanoparticles

A A D T Abeysinghe[1], N M S Sirimuthu[1]*, P P R Perera[2]
[1]*Department of Chemistry, University of Sri Jayewardenepura, Sri Lanka*
[2]*Department of Biochemistry, University of Sri Jayewardenepura, Sri Lanka*
**Email: nmssirimuthu@sjp.ac.lk*

Obesity is a serious health issue related with increased body fat content. Escalating numbers of patients have been reported worldwide throughout the recent past, associated with a high cost in the healthcare sector. Other than being a disease condition by itself, it also acts as a risk factor for many metabolic and cardiovascular diseases. There is no definitive treatment available and control of the disease is achieved via lifestyle modifications; hence, early detection of the risk to be obese is of paramount importance. Nevertheless, accurate diagnostic methods for obesity are not widely available in the current clinical setting due to the high cost and associated drawbacks. Utilization of 'leptin', which is an accurate indicator of body fat content, has gained the attention of researchers as a biomarker for obesity.[1] Therefore, this study was conducted with the aim of developing a novel immunoassay for the detection of leptin; a biomarker for obesity.

Leptin detection was done using an immunoanalytical method by surface functionalization of silver nanoparticles using anti-leptin antibodies.[2] Silver nanoparticles were synthesized by reduction of silver nitrate using sodium borohydride. Prepared silver nanoparticles were characterized using UV-Vis spectroscopy, dynamic light scattering (DLS) and scanning electron microscopy (SEM). The SPR peak was found to have a λ_{max} of 405 nm with a FWHM of 72 nm and the average particle size was recorded as 40 nm. Bovine serum albumin (BSA) was used to stabilize the synthesized silver nanoparticles sterically and the optimum BSA concentration required was found to be 10 µg/ml. Synthesized nanoparticles were surface functionalized using anti-leptin antibodies which specifically bind with leptin. These antibody-nanoparticle conjugates were characterized by a currently used immunoassay technique named Enzyme Linked Immunosorbent Assay (ELISA), UV-Vis spectroscopy and SEM and corresponding data verified the successful functionalization. Optimum pH and antibody-nanoparticle ratio for this functionalization process were determined using ELISA and according to obtained results, pH 9.5 and 1:10 ratio were selected to be the best conditions. Detection principle of this novel assay was based on the immuno-aggregation of anti-leptin functionalized silver nanoparticles in the presence of leptin. Changes in surface plasmon resonance due to this leptin induced aggregation were manifested via UV-Vis spectroscopy and spectral changes in the absorption peak confirmed the leptin detection ability.

This nanoparticle based detection system could be used as an intermediate detection step for qualitative analysis of samples as positive or negative for leptin. It could be further developed as a novel method to measure body fat content thereby allowing the early diagnosis of the risk towards obesity. This study gives insight to a promising alternative method to existing detection methods which are more expensive and time consuming.

Keywords
Obesity, leptin, Silver nanoparticles, anti-leptin antibodies

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